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Fate of Trace Elements in Sewage Sludge-Amended Soils

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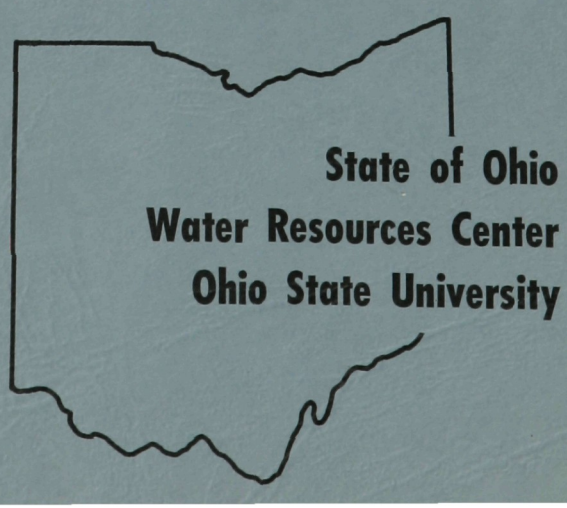

State of Ohio
Water Resources Center
Ohio State University

FATE OF TRACE ELEMENTS IN SEWAGE SLUDGE-AMENDED SOILS

Office of Water Resources Research and Technology
Department of the Interior
Project No. B-065-OHIO
Agreement No. 14-0001

This Final Report Covers Work Done Between
July 1, 1975 and March 31, 1978

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INTRODUCTION AND SUMMARY

Beginning 1 July 1975, three University of Dayton professors, Dr. Charles J. Ritter of the Geology Department, Dr. Charles R. Cothern of the Physics Department and Dr. Edward E. Zamierowski of the Biology Department, received a two-year matching grant from the Office of Water Resources Research and Technology (OWRT), Department of the Interior and the University of Dayton. The project was entitled "Fate of Trace Elements in Sewage Sludge-Amended Soils" (Project Number B-065-OHIO, Agreement No. 14-0001). The project had the following objectives:

(1) To analyze and characterize several of the major soil types and sewage sludges from urban and rural localities in the Dayton, Ohio, area
(2) To determine the chemical forms (compounds) of trace elements in sewage sludge and soil
(3) To determine the uptake and translocation of trace elements by plants grown on untreated and sewage sludge-amended soils
(4) To determine the nature of downward movement in soil of trace elements derived from sewage sludge

This report contains one published paper, one Master of Science (Biology) thesis, and seven drafts of papers which have been or will be submitted for formal publication. These papers describe our efforts to fulfill the objectives and the results and conclusions of the studies. The following is a brief summary of the techniques used and the conclusions reached.

Soils and sewage sludges were analyzed by atomic absorption spectroscopy for trace amounts of cadmium, copper, lead, nickel, and zinc. The city of Dayton sludge contains substantial amounts of these elements compared to that from other industrial cities. Sludge from Englewood, Ohio, a small non-industrial city to the north of Dayton has, for the most part, considerably smaller amounts of the trace heavy metals.

Isopycnic zonal centrifugation, using tetrabromoethane-ethanol density gradients, was used to fractionate sewage sludge and soil. Up to 75 weight percent of sludge has relatively low density (1.60 - 2.10 gm/cm^3). Eighty weight percent of soil has relatively high density (2.50 - 2.90 gm/cm^3). Organic matter makes up a large portion of low density material. Seventy-five weight percent of the total trace elements in sludge are contained in organic-rich density fractions. In soil there is a strong positive correlation between trace element concentrations and organic matter content. A relatively small amount of the trace elements is associated with inorganic compounds, particularly sulfides and sulfates.

Considering data from electron spectroscopy for chemical analysis (ESCA), it is concluded that zinc and copper are compounded with oxygen in sludge. Since it is chemically similar to zinc, cadmium is probably also bonded to oxygen. Because of the association of trace elements with organic matter, it is proposed that the heavy metals are, for the most part, in organic compounds. Infrared spectroscopy suggests carboxyl group compounds as the most likely hosts for the trace heavy metals.

Sugar beets and spinach were grown in untreated and sludge-amended Miami silty loam, Fox sandy clay loam and Ross sandy clay loam using Dayton and Englewood sludges. Amounts of sludge applied to soils were calculated using a formula proposed by the Ohio Agricultural Research and Development Center. Considering various aspects of the soil and sludge, maximum and fractions of the maximum allowable amounts of sludge were used. Plants were grown in controlled growth chambers for 50 days.

Addition of any amount of Dayton sludge reduced sugar beet and spinach yields, whereas added Englewood sludge increased plant shoot dry weight of both plant species. Application of the maximum allowable amount of Dayton sludge all at once reduced yield more than incremental additions of the same amount of sludge.

The pattern of plant heavy metal uptake, which differs for each metal, is complicated by interactions among soil type, plant species and plant part. Addition of any amount of Dayton sludge caused uptake of zinc to be greater in shoots and roots of both plant species grown in the three soils, than in control plants. No other metal uptake was this extensive. Englewood sludge did not increase any single metal uptake in both plant species and in all soils. In fact, neither plant species accumulated more cadmium or lead than controls in any soil.

Available cadmium, as extracted with DTPA from soil amended with Dayton sludge, has the strongest negative correlation with plant dry weight for both species. The strongest negative correlations between dry weight and plant heavy metal content is with cadmium for spinach and with copper for sugar beets. After cadmium, lead in the soil explains the greatest amount of variation in dry weight. Soil cadmium also strongly correlates with most heavy metal contents in plants. Copper strongly correlates with some plant metal contents, particularly those in the roots.

Because of the great interest in cadmium that exists today, more work was done with this element than any of the others. Primary (undigested) Dayton sewage sludge was enriched with cadmium and anaerobically digested in the laboratory. The object was to place a large concentration of cadmium (1413 ppm) in the same chemical form that naturally occurs in sludge. It was found that relatively less cadmium could be extracted by DTPA from this material than from that to which cadmium had been added after digestion. Therefore, less cadmium was available for plant uptake with the enriched, lab-digested sludge.

Cadmium concentrations in sugar beet roots increase with increasing amounts of Dayton sludge applied to Miami soil. Cadmium concentration in sugar beet roots was in all cases greater than in shoots of plants. It was also found that cadmium was more available (DTPA extractable) in soil after crop harvest than before planting. Therefore, it appears that cadmium is released from sludge and becomes increasingly available for plant uptake during crop growth.

Phosphate addition to Fox sludge-amended soil reduced sugar beet shoot Zn concentrations but did not consistently affect uptake of other metals. Possible synergisms between extractable Cu and Zn, Cd and Pb uptake by beet roots suggest that addition of sludge low in Zn, Cd and Pb but high in Cu to soils cropped with root vegetables may increase the amount of Zn, Cd and Pb in the human food chain.

Primary sludge was also enriched with ^{109}Cd and anaerobically digested in the laboratory. The sludge was applied to Miami soil on which sugar beets were grown. Cadmium accumulation sites and the path of cadmium transport in plants

were determined by Radioautography. Leaves of plants grown in ^{109}Cd-labeled sludge-amended soil show cadmium accumulation to occur in the midrib > principal lateral veins > leaf margin > mesophyll.

Sludge was applied to the tops of soil columns in the laboratory, and the systems were watered to simulate natural rainfall. The columns were separated into layers and analyzed for heavy metals. In the Miami (high clay content) soil, heavy metal ions did not penetrate below 15 cm. In the Fox (high sand content) the ions did not penetrate below 40 cm. It is believed that amendment of Dayton sludge to local farmlands does not pose a serious threat to ground water purity.

COMPARISON OF TRACE HEAVY METAL VALUES IN SEWAGE SLUDGE FROM DAYTON, OHIO, AND ENGLEWOOD, OHIO, WITH OTHER CITIES

A. G. Page (1974) has done an extensive literature review for the Environmental Protection Agency entitled "Fate and Effects of Trace Elements in Sewage Sludge When Applied to Agricultural Lands." He points out that there is wide variation of total trace element concentrations of sewage sludges from about 300 treatment plants in the United States, Canada, England, Sweden, and Wales. Table 1 shows the ranges for five elements. The levels of metals in Dayton sludge fall within these ranges. Furr et al., (1976) surveyed sewage sludges from 16

TABLE 1.

TRACE METAL CONCENTRATION (ppm)
(from Page, 1974)

	Zn	Pb	Ni	Cd	Cu
Ranges from 300 treatment plants in the United States, Canada, Sweden, England, and Wales (Page, 1974)	72 - 49,000	15 - 26,000	10 - 5,300	1 - 1,500	52 - 11,700

American cities during 1972 to 1973 for 68 elements, including the five we have studied. Table 2 gives the values for these elements. A comparison of the Dayton values with those of the other cities shows that: the Dayton Cd value (414 ppm) is exceeded only by that for Milwaukee (443.7 ppm); the Dayton Cu value (2,409 ppm) is exceeded only by that for Los Angeles (2,890 ppm) and that for Philadelphia (2,680 ppm); the Dayton Ni value (575 ppm) is higher than any of the cities, Denver (562 ppm) is comparable; the Dayton Pb value (3,080 ppm) is exceeded only by that for Philadelphia (7,627 ppm), Los Angeles (3,065 ppm) is comparable; and the Dayton Zn value (10,030 ppm) far exceeds those for any of the cities, the closest value is for Philadelphia (6,890 ppm).

TABLE 2.

TRACE METAL CONCENTRATIONS (ppm)
From Furr, et al., (1976)

	Cd	Cu	Ni	Pb	Zn
Atlanta, GA	104.2	1,463	169	1,445	2,838
Cayuga Hts., NY	6.8	821	36.4	136	560
Chicago, IL	14.8	578	51.2	605	1,160
Denver, CO	46.1	1,370	562	1,011	2,860
Houston, TX	111.8	1,560	102	2,236	2,560
Ithaca, NY	66.7	1,300	166	329	1,700
Los Angeles, CA	171.4	2,890	402	3,065	4,590
Miami, FL	149.6	1,200	453	1,467	1,400
Milwaukee, WI	443.7	1,288	360	2,253	1,370
New York, NY	29.7	1,890	140	1,976	1,340
Philadelphia, PA	192.4	2,680	432	7,627	6,890
San Francisco, CA	8.5	900	223	2,521	601
Schenectady, NY	22.4	900	72	598	1,090
Seattle, WA	64.0	1,170	153	2,411	1,830
Syracuse, NY	200.0	1,060	211	1,412	1,840
Washington, D.C.	31.0	458	No Value	498	1,475
Dayton, OH	414.0	2,409	575	3,080	10,030
Englewood, OH	6.2	863	1825	221	965

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UPTAKE AND DISTRIBUTION OF CADMIUM IN *BETA VULGARIS* (SUGAR BEET) FROM SLUDGE-AMENDED SOIL

Thesis

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by

Peter Harris Brown, B.A.

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Dayton, Ohio

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ABSTRACT

UPTAKE AND DISTRIBUTION OF CADMIUM IN BETA VULGARIS (SUGAR BEET) FROM SLUDGE-AMENDED SOIL.

Peter Harris Brown, B.A.
University of Dayton, 1977.

Major Professor: Donald R. Geiger, Ph.D.

Municipal sewage sludge presents a serious disposal problem for many urban communities. To assess the feasibility of utilizing municipal sewage sludge as an agricultural soil amendment, a study was initiated to evaluate plant growth and cadmium uptake, and cadmium availability in soil treated with an anaerobically digested sewage sludge.

Sewage sludge obtained from Dayton, Ohio, was applied to Miamian silt loam, an important agricultural soil in Montgomery, County, Ohio, in amounts equal to 0, 2.51, 5.02, 12.55 and 25.10 dry tons/acre. Sugar beets (Beta vulgaris, L.) were planted in sludge-amended soils, grown in the laboratory for 50 days, and harvested. After harvesting the first crop, sludge in the amounts equal to 2.51 and 5.02 tons/acre were again added to those soils which initially received sludge in the amounts equal to 2.51 and 5.02 tons/acre, respectively. A second crop of sugar beets was then planted in all soils. This procedure continued for five crops such that the fifth crops grown in the soils given sludge in the amounts of 2.51 and 5.02 tons/acre crop^{-1} were exposed to the same amounts of sludge as the first crops grown in the soils initially given 12.55 and 25.10 tons/acre, respectively. Shoot and root yield and heavy

metal concentration, and available (DTPA-extractable) soil heavy metals were determined for each crop.

Anaerobically digested ^{109}Cd-labeled sludge was produced in a laboratory designed digester. The ^{109}Cd-labeled sludge was applied to Miamian silt loam on which sugar beets were grown to determine cadmium accumulation sites and the path of cadmium movement in plants.

The data suggests that Dayton sewage sludge should not be utilized as an agricultural soil amendment. Both the amount of Dayton sludge applied and the mode of sludge application to soil affected the yield of crops. Addition of sludge to soil in the amounts equal to 12.55 and 25.10 tons/acre decreased crop yield, and initially adding the sludge to soil all at once reduces yield greater than incremental additions of the same amount of sludge.

Cadmium concentration in roots increases with increasing amounts of sludge applied to soil. Cadmium concentration in roots was always greater than in shoots of plants grown in sludge-amended soils. Cadmium concentration in shoots increases with increasing plant age and leaf age. Radioautographs of plant leaves of plants grown in ^{109}Cd-labeled sludge-amended soil show cadmium accumulation to occur in the midrib > principal lateral veins > leaf margin > mesophyll. Passive uptake of cadmium from soil, and translocation of cadmium in the transpiration stream coupled with sequestration of cadmium ions by pectin in the leaf free space may explain the observed pattern of cadmium accumulation in plants.

Soil extractable cadmium increases with increasing amounts of sludge added to soil, and with successive cropping of sludge-amended

soils. The majority of the potentially available sludge-borne cadmium is available to crops planted immediately following sludge application to soil. This may suggest that the majority of potentially available sludge-borne cadmium is complexed with simple organic compounds.

Multiple stepwise regression analysis of DTPA-extractable soil heavy metals and heavy metal uptake by plants show a positive correlation between extractable soil cadmium and the uptake of zinc by plants, and positive correlations between extractable soil zinc and the uptake of copper, nickel and lead by plants.

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I am deeply grateful to Drs. Edward E. Zamierowski, Charles J. Ritter and C. Richard Cothern for their guidance on this project and particularly for their support and encouragement. Special appreciation is extended to Dr. Donald R. Geiger for his technical help and criticisms. I would also like to thank Steve Bergman for his technical help and Donald Roman, technical supervisor of the Dayton Municipal Wastewater Treatment Plant, whose cooperation made this project possible.

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INTRODUCTION

Disposal of sewage sludge from industrial centers is a growing problem for municipalities. Current methods for sewage sludge disposal are incineration, ocean dumping and land disposal in sludge lagoons or as landfill. Incineration of sewage sludge is impractical due to the high cost of fuel and its contribution to air pollution (Walker, 1974). Since ocean dumping of sewage sludge may only be practical for a few coastal cities, the only practical alternative for the safe disposal of sewage sludge is land disposal.

The high cost and limitation of urban property has made lagooning prohibitive. Sludge lagooning within city limits also presents potential health and water pollution hazards. Disposal of sewage sludge as landfill outside city limits may not be practical due to the lack of suitable sites, and as sites are filled, other means of sewage sludge disposal will be necessary.

The chemical characteristics of sewage sludge combined with the rising costs of fertilizer have prompted investigations of using sewage sludge as an agricultural soil fertilizer and conditioner. Sewage sludge has a large amount of organic matter and contains the major nutrients, nitrogen, phosphorus and potassium, needed by plants. Sewage sludge also contains appreciable amounts of micronutrients, especially zinc, copper, magnesium, manganese, iron, boron, cobolt, and molybdenum. In small quantities, these micronutrients are also essential for normal

plant growth.

In addition to plant nutrients, industrial sewage sludge contains varying amounts of salts, pathogenic microorganisms, persistent and carcinogenic chemicals (e.g., dieldren, polychlorobiphenyls) and heavy metals (Furr et al., 1976b).

Prior to the initiation of extensive programs of land application of sewage sludge, the potential environmental hazards must be evaluated. These hazards include the contamination of the soil environment and ground water with potentially toxic substances or pathogens, and the increased incorporation of toxic substances into the food chain with subsequent harmful effects on plants, animals, and ultimately, man. In this study, some of these hazards associated with the application of Dayton Municipal sewage sludge to an important agricultural soil in Montgomery County, Ohio were studied. In particular, the incorporation of the heavy metal cadmium into the food chain from sludge-amended Miamian silt loam was studied.

Objectives of this Study

1. Can Dayton sewage sludge be utilized as a fertilizer or soil conditioner to increase yield of sugar beet plants grown on Dayton sludge-amended Miamian silt loam?
2. What are the effects on the uptake of cadmium by sugar beet plant tissue when Dayton sludge is applied to Miamian silt loam in amounts up to the maximum recommended by the Ohio Agricultural Research and Development Center?
3. What are the effects on cadmium uptake by successive crops of sugar beets by application of equal amounts of Dayton sludge to Miamian

silt loam using different modes of application (initial vs. incremental addition of the total amount of sludge)?

4. Investigate available cadmium, as measured with the extractant DTPA, in sludge-amended soil and the effects of different modes of application (initial vs. incremental additions) of Dayton sludge to Miamian silt loam on the amount of extractable cadmium.
5. Determine whether successive croppings of Dayton sludge-amended Miamian silt loam with sugar beet plants affects the amount of soil extractable (DTPA) cadmium.
6. Determine the site(s) of cadmium accumulation in sugar beet plants.
7. Look for correlations between heavy metals (cadmium, zinc, nickel, copper and lead) available in soil and heavy metal uptake by sugar beet plants grown in Dayton sludge-amended Miamian silt loam.

LITERATURE REVIEW

Land disposal of sewage sludge has the advantage of being less expensive than other disposal methods, and may be considered as the ultimate disposal method (Black and Kronis, 1973). The heavy metal content of industrial sewage sludge has caused concern over possible entry of heavy metals into the food chain and their toxicity to crops when sewage sludge is applied to agricultural land. Cadmium is one of these heavy metals and cadmium accumulation by crops is of particular concern because it has been shown that excessive amounts of cadmium in food adversely affects the health of humans (Nordberg, 1974).

Estimation of the daily intake of cadmium ranges from 25-60 μg/day for man (Nordberg, 1974). This estimation is based on data of cadmium concentrations in food of several countries (United States, West Germany, Czechoslovakia, Rumania and Japan). The estimated range of daily human cadmium intake may be compared with the maximum tolerable daily intake of 70 μg/day for man proposed by the joint FAO/WHO expert committee on food additives (Nordberg, 1974). It is therefore imperative to keep cadmium levels in food from rising. Small increases in crop accumulation of cadmium may be insufficient to cause noticeable detrimental effects to crops, yet still cause increased accumulation of cadmium by animals and man.

Nordberg (1974) lists abnormalities associated with chronic cadmium accumulation in man from foods: Itai-Itai Disease (Renal Osteomalacia);

anemia; hypertension and associated cardiovascular disorders; renal tubular dysfunctioning. Absorbed cadmium accumulates in the kidney due to its slow excretion from the body (Stenstrom and Lonsjo, 1974) and is selectively accumulated in the tubules of the kidney cortex (Nordberg, 1974). Furst (1971) states that the metallic powder, oxide and sulfide of cadmium has been shown to be carcinogenic. In mice, hamsters and rats, teratogenic effects of cadmium have been observed (Hiatt and Huff, 1975).

Chemistry of Sewage Sludge

Page and Chang (1975), in an extensive review of trace metals in sewage sludge, point out that there is a wide variation in concentration of heavy metals in sewage sludges analyzed from more than one hundred and twenty treatment plants throughout the U.S.A. Ranges of heavy metals and of nitrogen, phosphorus and potassium found in sewage sludges are listed in Table 1. Organic matter content of sewage sludge ranges from 40-70 percent on a dry weight basis, the remainder being composed of sand, silt and other inorganic wastes (Menzies, 1973).

Chaney (1973) states that the basis for recommendation of applying sewage sludge containing heavy metals to agricultural soils is the benefit:risk ratio. Potential benefits of applying sewage sludge to land listed by Volk (1975) are: inexpensive nutrient source for nitrogen, phosphorus, potassium, zinc, copper, boron and molybdenum for farmers; improved soil structure and moisture retention; increased soil temperature; erosion control; increased soil organic matter and cation exchange capacity; a practical, inexpensive method for disposal of sewage sludge. Potential risks are: hazards of increased bioaccumulation of trace

Table 1. Trace element concentration and percent major plant nutrients in sewage sludges from more than one hundred and twenty treatment plants throughout the U.S.A.*

Element (total)	Range in sludge (dry weight basis)	Median in sludge (dry weight basis)
	%	%
N	1-15	2.0
P	1-6	1.3
K	0.05-1	0.2
	PPM	PPM
Cd	1-1,100	10
Cr	20-33,000	400
Cu	100-11,700	700
Hg	0.1-50	3
Mo	2-1,000	5
Ni	10-4,500	50
Pb	10-26,000	500
Zn	100-28,500	2,200

*From Page and Chang, 1975.

metals by plants and their subsequent entrance into the human food chain; water contamination due to the percolation or surface runoff of trace elements and nutrients, especially nitrate; plant and animal pathogens; persistent and carcinogenic chemicals; crop restrictions; odors; sociological acceptance; decreased productivity of crop lands; rendering crop lands unsafe for continued general farming.

The first and last two risks are primarily a function of heavy metal pollution of agricultural soils. Increased cadmium in the human food chain may result from growing crops on soils amended with sewage sludge (Furr et al., 1976a). Decreased productivity of crop land may be the result of toxic effects of heavy metals, especially zinc, copper and nickel, on plants (Chaney, 1973). Rendering of crop lands unsafe for continued general farming would result from excessive heavy metal loading of soils. This consequence may not be immediately manifested following sewage sludge application to soil, but may occur after the added organic matter has been degraded and an equilibrium between heavy metals, phosphate and soil colloidal micelles has been established.

Table 2 represents reasonable and attainable heavy metal concentrations for digested sewage sludge. Sewage sludge meeting the criteria in Table 2 would have a good benefit:risk ratio with respect to heavy metals for application on agricultural soils (Chaney, 1973). He further states that sewage sludge which does not meet the criteria of Table 2 should not be applied to agricultural land.

Many guidelines for the application of sewage sludge to agricultural soils have been recommended. The Ohio Agricultural Research and Development Center (O.A.R.D.C.) has proposed a formula whereby the maximum amount of sewage sludge which may ever be applied to agricultural

Table 2. Heavy metal concentrations of sewage sludge appropriate for agricultural land application.*

Element in sewage sludge	Total μg/g sludge (dry weight basis)
Cd	0.5% of Zn
Cu	800
Hg	15
Ni	100
Pb	1,000
Zn	2,000

*From Chaney, 1973.

soils may be calculated (Grooms, 1975). Maximum permissible application rates of sewage sludge are calculated by the following equations:

Equation 1.

$$\text{Total amount of sludge (dry tons/acre)} = \frac{\text{C.E.C. X 32,700}}{\text{ppm Zn + 2(ppm Cu) + 4(ppm Ni) - 200}}$$

Equation 2.

$$\text{Total amount of sludge (dry tons/acre)} = \frac{\text{C.E.C. X 16,350}}{\text{ppm Zn + 2(ppm Cu) + 4(ppm Ni) - 200}}$$

C.E.C. = cation exchange capacity of the unamended soil in milliequivalents/100 grams soil.[1]
ppm of heavy metals are determined from total analysis of dry sludge.

In the equations, C.E.C. of a soil is a measure of a soil's capacity to adsorb cations reversibly, such that the cations adsorbed by soil colloidal micelles may be substituted by cations in the soil solution. Equation 1 should be used for soils where pH can be maintained at 6.5 or greater. In those regions where soil pH is lower than 6.5, equation 2 adjusts for greater availability of heavy metals in soils which are acidic. The denominator of the equations is in "zinc equivalents" due to phytotoxicity of zinc on plants above certain concentrations of zinc in plants. Copper and nickel concentrations in sludge are critical because copper is twice and nickel four times as toxic to plants as zinc (Grooms, 1975); other heavy metals are not considered important in this toxicity index. The minus 200 in the denominator adjusts for addition to the soil of some exchange capacity in the inorganic constituents of applied sludge (Chaney, 1974).

Cadmium is a special problem in sewage sludge. The maximum amount of cadmium relative to zinc that should be allowed in sewage sludge

[1]See Apendix B for further explanation.

applied to agricultural soils is determined by the Cd:Zn ratio. If the cadmium concentration in sewage sludge is less than or equal to 0.5% of the concentration of zinc, accumulation of levels of cadmium in plants which are toxic to humans and animals will be prevented (Chaney, 1973). This is because prior to such cadmium accumulation, phytotoxicity resulting from zinc poisoning will severely damage crops (Keeney et al., 1975).

Factors Affecting Cadmium Availability in Soils

Background levels of cadmium in uncontaminated soils vary between 0.01-0.7 ppm, although most soils contain less than 0.1 ppm cadmium (Rohde, 1974). Major sources of pollution which may increase cadmium levels in soils are fallout, particularly from smelters, irrigation with contamined water, attrition of automobile tires, combustion of automobile fuels, pesticides, herbicides, fungicides, fertilizer and sewage sludge. (Sanjour, 1974; Hemphill et al., 1973).

Availability of heavy metals in soils is determined by a soil's physical and chemical properties and biological characteristics (Table 3). Physical properties of soils which may affect the availability of heavy metals in soils are soil texture, structure, moisture, and temperature; and physical properties may modify or be modifed by chemical properties and biological characteristics of a soil.

Soil texture, the relative proportions of different size separates (sand, silt and clay), strongly influences the rate and extent of many physical and chemical reactions in soils because texture determines the amount of surface on which reactions can occur. Water available to plants, capillary water, is held as a film on the surface of clay particles. As fineness of texture increases, there is a general increase

Table 3. Physical and chemical properties and biological characteristics of soils affecting cadmium availability in soils.

Properties	Soil factor	Increases cadmium availability in soils	Decreases cadmium availability in soils
Physical Properties	Texture	Increasing fineness of texture (to a limit) retaining granulated structure	Decreasing fineness of texture
	Moisture and air relationships	Large moisture percentage causing reducing conditions	Aerobic conditions or decreasing soil water below the wilting coefficient
	Temperature	High	Low
Chemical Properties	pH	Increasing acidity	Increasing alkalinity
	Cation exchange capacity	Low	High
	Exchangeable bases	Small amounts not greatly increasing soil pH	Large amounts which increase soil pH
	Available phosphate	Large amounts	Small amounts
Biological Characteristics	Organic matter	Large amounts of Type-I complexes and small amount Type-II complexes	Large amounts of Type-II complexes and small amount of Type-I complexes
	Organisms	Large number of those which release into the soil acids, Type-I complexes, CO_2	Large number of those which concentrate cadmium, increase soil pH or release Type-II complexes

in available water storage, although clays frequently have a smaller water-storing capacity than do well granulated silt loams due to their poor soil structure relative to silt loams. The negative charged surface allows clay minerals to adsorb cations from the soil solution onto their exterior surface or close enough to their surface to retard ion movement out of the soil by leaching. These cations may be used by plants.

Soil structure, the aggregation of primary soil particles, may modify the influence of soil texture with regards to moisture and air relationships, action of microorganisms, root growth, amount of surface area available for chemical and physical reactions and availability of nutrients and heavy metals to plants.

Soil moisture may affect availability of heavy metals in soils. Flooded soils are under reducing (anaerobic) conditions. It is thought that reducing conditions in soils slows down or inhibits reversion (the process by which heavy metals in soils become fixed with time to chemical forms less available to plants). Reducing conditions in soils increases the availability of nickel compounds (Rohde, 1974). The amount of soluble manganese in a solution of clay colloids under reducing conditions was considerably more than the amount found soluble under aerobic conditions (Reddy and Perkins, 1974). Nambiar and Cottenie (1971a) observed that an increase in soil moisture within ranges that permit aerobiosis, resulted in reduced extractable (with NH_4OAc) manganese. The amount of extractable (with NH_4OAc) manganese and iron found in soils under reducing conditions was greater than found in soils under nonreducing conditions (Nambiar and Cottenie, 1971b).

When soil moisture content becomes extremely low, as in the case of drought, availability of heavy metals may be reduced. When the moisture

content falls below the wilting coefficient (15 bars), the rate of transference of divalent cations through the soil matrix is negligible (Nambiar, 1976).

Soil temperature may indirectly affect availability of heavy metals in soils, and is influenced by soil color, moisture and incident solar energy. At low soil temperatures (0-15°C), rates of biological decomposition of organic matter are slow, thereby limiting the rate at which nutrients and heavy metals are made available from organic matter. In addition, rates of plant root metabolic processes, such as respiration, are reduced at lower soil temperatures. Lowering the rate of root metabolic processes reduces the rate of root growth and accumulation of nutrient and non-nutrient ions. MacMillan and Hamilton (1971) observed that increasing soil temperature increases both copper and manganese concentrations and uptake by carrots regardless of whether the source of the respective nutrient was native or applied.

Chemical properties of soils which influence the availability of heavy metals in soils are pH, cation exchange capacity, exchangeable bases and available phosphate. Heavy metals become much more available as the soil becomes increasingly acidic (Chaney, 1973) because increasing the concentration of hydrogen ions in the soil solution increases the probability of hydrogen ions substituting for heavy metal ions on exchange sites of soil colloids. As heavy metal ions become more concentrated in the soil solution, they too may substitute for cadmium and other metal ions which may be loosely bound on the soil's clay mineral, sesquioxide and humus fractions. The process whereby cations in the soil solution may exchange for cations bonded to soil colloidal particles is known as cation exhange.

The cation exchange capacity (C.E.C.) of a soil is a measure of the sum total of exchangeable cations that a soil can adsorb. The term "exchangeable cations" is rather vague, and the C.E.C. of a given soil is, to a large extent, dependent upon the method used to determine it. Fine textured soils tend to have a larger C.E.C. than sandy soils. Within a given textural group, organic matter and the amount and type of clay minerals influence the C.E.C. Generally, availability of heavy metals in soils decreases with increasing C.E.C. (Chaney, 1973).

Several factors operate to expedite or retard release of heavy metals from cation exchange sites on soil colloids. First, there is the proportion of the C.E.C. of a soil occupied by the given cation in question. For example, the greater the percentage of calcium saturation of a soil, the greater is the ease and rapidity with which calcium ions are desorbed from cation exchange sites (Brady, 1974). A second important factor influencing exchangeability of cations from cation exchange sites is the effect of ions held in association with them. The limitation of availability of a given cation from cation exchange sites as a consequence of ion association is exemplified by the decreased magnesium availability to plants as a result of excessive quantities of exchangeable potassium (Brady, 1974). Thirdly, exchangeability of cations from cation exchange sites is influenced by the tenacity with which different colloidal micelles hold specific cations. For example, at a given percentage base saturation, the strength with which calcium is held by montmorillonite is much greater than that held by kaolinite (Brady, 1974).

Soils containing large concentrations of exchangeable bases (alkali and alkaline earth metals, principally calcium, magnesium, potassium and sodium) tend to be alkaline. This is because cation exchange sites on

soil colloidal micelles are saturated with these cations due to mass action. Thus, hydrogen ions will remain in the soil solution and percolate through soil with the water instead of bonding with cation exchange sites on colloidal micelles. Calcareous soils, such as Miamian silt loam, contain carbonates of calcium and magnesium. These salts of a moderately strong base ($CO_3^{=}$) and a weak acid generally give calcareous soils their characteristic alkaline reaction. The carbonate-buffered alkaline pH of most calcareous soils keeping metal ions insoluble may be the mechanism which reduces heavy metal availability in these soils (Dowdy and Larson, 1975a).

Phosphate ions in the soil solution may reduce heavy metal uptake by plants. Chaney (1974) found that phosphate decreases stunting of plant growth caused by excessive levels of zinc, copper and nickel and that phosphate strongly counteracts metal toxicity. The suggested mechanism by which phosphate reduces heavy metal availability in soils is bonding of phosphate ions with heavy metal ions. Since all normal phosphates of metals are quite insoluble, except for those of alkali metals (Eshbach, 1966), the phosphates of heavy metals would precipitate out of the soil solution. Precipitation of cadmium phosphates out of the soil solution would prevent cadmium ions from binding to sites on plant roots and their subsequent uptake by plants (Culter and Rains, 1974).

The effect of available soil phosphate on the availability of cadmium to plants appears to be opposite of its effect upon the availability of other heavy metals. Increasing available soil phosphate may increase cadmium availability to plants (Miller et al., 1976; Williams and David, 1976 and 1977), although why this occurs is not known.

Biological characteristics of soil affecting availability of cadmium in soils are organic matter and soil organisms. Organic matter is a mixture of plant, animal and microorganismal exudates and remains, and products of their decay (humus). Organic matter is the major agency in the encouragement and stablization of granular-type aggregates in surface soil horizons. Increased soil granulation increases soil porosity, which in turn increases soil aeration and soil water holding capacity at field capacity. Organic matter has a high water holding capacity, but its wilting coefficient is also proportionally high. Thus, the net direct contribution of organic matter towards soil's available water holding capacity is of less consequence than the effect of organic matter on soil granulation, thereby indirectly increasing the available water holding capacity of a soil.

Chaney (1973) attributes adsorption of exchangeable heavy metals by organic matter to the ability of organic matter to chelate heavy metal ions. The organic matter's ability to retain heavy metal ions depends on the bond strength of the adsorbed heavy metal ion, which in turn is dependent upon the particular cation bonded and the particular molecule and electron-donor functional group to which the cation is bonded.

In an investigation to determine the amount of cadmium adsorbed by thirty different soils, John (1971) concluded that the amount of cadmium adsorbed by soils increased as their organic matter and C.E.C. increased. In a study carried out on four hundred and fifty three soils, MacLean and Langille (1976) showed a positive correlation between soil organic matter and extractable (0.1 N HCl) soil zinc. Soldatini et al. (1976) observed that organic matter and clay content were the two dominant constituents of soil contributing towards lead adsorption in soils. Karim and

Sedberry (1976) reported a highly significant ($P \leq .01$) positive correlation between DTPA-extractable zinc and organic matter in Ap horizons of alluvial soils of Louisiana. Karim et al. (1976) reported highly significant ($P \leq .01$) positive correlations between organic matter content and DTPA-extractable copper in alluvial soils of Louisiana.

Kirkham (1977) states that in most cases, heavy metal micronutrients are so insoluble, that in the absence of humus it is impossible for crops to obtain enough heavy metal micronutrients for normal plant growth. Zunino and Martin (1977) have proposed a model for the involvement of soil organic matter in the translocation of metal ions from rocks to biological systems. They hypothesize that soil organic matter is composed of three types of organic complexants (organic molecules which may bond with metal ions and form complexes) and that heavy metal translocation includes a sequence of reactions involving increasing affinity between metal ions and electron-donor organic molecules. Type-I complexants, excreted by pioneering organisms (e.g., lichens), plant roots and soil microorganisms, are simple soluble molecules such as amino and other organic acids. Type-II complexants are insoluble organic molecules with numerous functional groups which sequester metal ions from Type-I complexes and form Type-II complexes which are of greater stability than Type-I complexes. Type-II complexants are derived from microbes which synthesize extracellular, insoluble, highly polymerized molecules which may contain any combination of carboxyl, phenolic and alcoholic hydroxyls, carbonyl, methoxyl, amino and azo functional groups. Type-II complexes become Type-III complexes when complete saturation of cation bonding sites on Type-II complexes is reached.

Increasing concentration and variety of Type-I complexants is a

result of biological activity and diversification and occurs continuously. As stability of the insoluble complexes decreases during transition from Type II to Type-III complexes, Type-I complexants present in the soil solution begin to successfully compete for metal ions associated with Type-III complexes. Thus, in mature soils, soluble Type-I complexes may be formed and present in great concentrations in the soil solution.

The relevance of this hypothesis to heavy metal availability to plants is that plants can incorporate soluble Type-I complexes into their cell structure (Zunino and Martin, 1977). They suggest that a possible mechanism for metal-ion absorption by root hairs could be the entrance through the cell membrane of Type-I complexes as such. These complexes would be transported through the plant sap to the location where they would be involved in a specific biochemical process. The similarity of complexants found in the soil solution and inside plant tissues and sap supports this hypothesis.

Soil organisms, especially bacteria, fungi, actinomycetes and higher plants, play an essential role in heavy metal availability in soils by their influence on soil structure, soil texture and their ability to concentrate and replenish heavy metals in soils. Plant roots promote granulation of soil by their decay in soil and their disruptive action as they penetrate through soil. The mechanism of humification involves organic tissue degradation, chemical and enzymatic polymerization reactions, cell autolysis, and microbial synthesis (Zunino and Martin, 1977). Microbial use of organic matter as an energy source also causes release of essential nutrient ions and heavy metals trapped in nitrogenous coordinate compounds into the soil solution. As a byproduct of respiration, many soil organisms release carbon dioxide into the soil

solution thereby causing an acidic reaction. As soil pH decreases, heavy metals are made more available. Some chemosynthetic bacteria are capable of producing nitrous, nitric, phosphorus, sulfurous or sulfuric acid which may also cause an acidic soil reaction.

Heavy metals may be absorbed and concentrated by soil microorganisms, hence decreasing their availability to plants. Soil organisms are also capable of secreting various organic compounds of which organic acids, extracellular enzymes and polysaccharides are examples. These organic molecules released into the soil solution may sequester heavy metal ions from soil colloids and/or the soil solution, and increase or decrease heavy metal availability depending upon the strength of the bond and from which soil fraction heavy metal ions were sequestered.

Accumulation of Cadmium by Plants

Cadmium uptake from soils and its distribution in plants has been studied by many investigators. The normal range of cadmium in plants is 0.2-0.8 ppm (Page, 1974). Plants grown in cadmium salt-enriched soils resulting in reduction of crop yield (John, 1972b; John et al., 1972b; Miller et al., 1977; Haghiri, 1973) and the increase of cadmium concentrations in plant tissues (Williams and David, 1977; Street et al., 1977; Hassett et al., 1976; John, 1973) have been well documented. Differential cadmium accumulation (Stenstrom and Lonsjo, 1974) and tolerances of plant tissues (John, 1972a; MacLean, 1976) and plant species (Page et al., 1972) of plants grown in cadmium polluted soils has also been reported. Cutler and Rains (1974) suggest that diffusion of cadmium into root cells, coupled with sequestration of cadmium on root cell walls, largely accounts for the accumulation and distribution of cadmium in barley plants grown in ^{109}Cd-enriched solution culture.

Addition of lime ($CaCO_3$, $Ca(OH)_2$ or CaO) to soils treated with cadmium salts decreases cadmium uptake by radish plants (John, 1972a and 1972b). Miller et al. (1976) observed positive correlations between soil extractable phosphorus (Bray P_1 Test) and cadmium concentrations in soybean plants. William and David (1976) observed increased cadmium concentrations in tops of oat plants when 250 ppm phosphate was added to a soil. Williams and David (1977) reported that effects of phosphate addition on cadmium uptake by plants varied from soil to soil. They observed that additions of phosphate to soil generally increased cadmium uptake by clover. On acutely phosphorus-deficient soils, increasing additions of phosphate decreased cadmium content of clover, but the decreased cadmium content was attributed to substantial increases in plant yield (Williams and David, 1977). They suggested that the level of available soil phosphorus may affect cadmium content of plants mainly through effects of phosphorus on plant growth and vigor.

The phytotoxicity of cadmium has been well demonstrated (John et al., 1972b; Williams and David, 1977) and its symptoms have been shown to be species specific. Cadmium toxicity begins to occur in soybean and wheat plants when cadmium concentration in soil enriched with $CdCl_2$ is 2.5 ppm (Haghiri, 1973). Cadmium toxicity in wheat plants appears as stunted growth (Haghiri, 1973; Huang et al., 1974), while in soybean, it resembles iron chlorosis (Haghiri, 1973). Cadmium toxicity in corn plants reduces growth. The critical concentration of cadmium in corn plants grown in solution culture, above which plants suffer from reduced growth, is about 20 ppm on a dry weight basis (Iwai et al., 1975). The threshold for depression of soybean dry weight lies in the range of 3 ppm to 5 ppm shoot cadmium concentration (Collins et al., 1976;

Haghiri, 1973; Miller et al., 1976). Sunflower plants show a 50% reduction in their rates of photosynthesis when cadmium levels in leaf tissue reach 93 ppm (Bazzaz et al., 1974). Strong negative correlations between net photosynthesis and leaf cadmium concentrations, and transpiration and leaf cadmium concentrations in excised sunflower tops have been demonstrated (Bazzaz et al., 1974). Cadmium has been demonstrated to inhibit activity of photosystem II in spinach chloroplasts (Li and Miles, 1975). Huang et al. (1974) reported that cadmium toxicity in soybean plants decreases the rate of photosynthesis and nitrogen fixation in the roots. Leaves analyzed from soybean seedlings grown in solutions containing approximately 0.15, 0.30 and 0.45 ppm cadmium (added as $CdSO_4$), showed increased activities of hydrolytic enzymes characteristic of a senescence response (Lee et al., 1975).

Factors affecting cadmium accumulation by plants grown on sludge amended soils have been investigated by many workers (Table 4). Yields and cadmium concentrations in plants grown on sludge-amended soils have been demonstrated to increase significantly compared to controls (Boswell, 1975; Dudas and Pawluck, 1975; Hinesly et al., 1977). Increasing amounts of applied sewage sludge causing increasing cadmium concentrations in plants has also been demonstrated (Andersson and Nilsson, 1974; Dowdy and Larson, 1975b). Addition of lime to increase soil pH has been shown to reduce cadmium uptake by plants grown in sludge-amended soil (Andersson and Nilsson, 1974; Linnman et al., 1973). Street et al. (1977) observed that cadmium content of plants grown on sandy soils amended with sludge was greater than plants grown on loamy and clay loam soils which had greater organic matter content and a greater cation exchange capacity. Bingham, et al. (1976) observed that cadmium concentrations in rice

Table 4. Factors affecting cadmium uptake by plants grown in sludge-amended soils.

Factor	Increase cadmium uptake	Decrease cadmium uptake
Soil	Factors which increase cadmium availability (Table 3)	Factors which decrease cadmium availability (Table 3)
Applied sludge	Increase amount applied	Decrease amount applied
Cadmium concentration in sludge	High	Low
Other heavy metals	Increasing Zn in sludges is correlated with > Cd uptake	
Time of planting relative to sludge application	Immediately following sludge application	Allowing incubation time of sludge-amended soil prior to planting

leaves increased with increasing cadmium concentrations in cadmium enriched ($CdSO_4$) sludge-amended soil.

Little is known about synergistic and antagonistic effects of various heavy metals with respect to cadmium uptake by plants grown in sludge-amended soils. Dowdy and Larson (1975b) observed a strong positive correlation between the concentration of zinc and cadmium in the edible portion of carrots and radish, and leaves of corn and lettuce. A positive correlation of leaf copper concentration on cadmium content of rice leaves and lettuce leaves was reported by Bingham et al. (1976) and Dowdy and Larson (1975b), respectively. A significant negative correlation of cadmium on zinc accumulation in leaf and edible tissues of field beans, soybean, corn, wheat and rice grown in cadmium-enriched ($CdSO_4$) sludge-amended soil was also reported (Bingham et al., 1975).

Hinesly et al. (1977) observed that sludge-borne cadmium incorporated into soil immediately prior to planting was the major determinant of levels of cadmium in leaf and grain tissues of corn when compared with amounts of cadmium supplied to the soil in sludge in previous years. Their data is in disagreement with the model of cadmium availability in sludge-amended soils as proposed by Leeper (1973). He suggests that sludge organic components complex metals reducing their availability for absorption by plants, and that phytotoxic conditions in soil may not occur until the organic matter has been decomposed by microbial processes some time after sludge applications have been terminated.

Summary

Data from various investigators suggest that the application of sewage sludge to agricultural land could produce concentrations of heavy

metals in soils which may increase or decrease crop productivity. In some cases where sludge application to agricultural soils increased crop productivity, crop quality, with respect to the concentration of cadmium, has decreased. Bioaccumulation of heavy metals by plants grown on sludge-amended soils depends upon the physical, chemical and biological characteristics of both soil and sludge, plant species grown on the sludge-amended soil, plant age, plant part, amount of sludge applied and length of time of incubation of the sludge-amended soil prior to cropping.

Recent awareness of the small margins of safety between existing human intake levels and levels of heavy metals which may cause undesired health effects in the population has caused concern over accumulation of toxic heavy metals, especially cadmium, in food plants.

Since further cadmium contamination of the food chain must be prevented and since many urban sludges which may be applied to agricultural lands contain large cadmium concentrations (e.g., Dayton sludge), it is imperative to determine the fate of sludge-borne cadmium before sewage sludge is applied to agricultural soils.

MATERIALS AND METHODS

Soil and Sewage Sludge Samples

The soil used in all experiments was Miamian silt loam. It is a moderately fine-textured, dark yellowish-brown, calcareous glacial till (United States Department of Agriculture, 1976). A representative surface sample (0-12 in) of Miamian silt loam, 0-2 percent slope, was obtained from $SE_{1/4}$ $SW_{1/4}$ sec. 13, T. 6N., R. 5 E., 725 feet west and 480 feet north of the intersection of Meeker Road and Frederick Pike, Butler Township, Montgomery County, Ohio, which was the site used in the Montgomery County soil survey (United States Department of Agriculture Soil Conservation Service, 1976). The soil was collected in plastic garbage cans and brought back to the laboratory in April, 1976, where it was dried and homogenized.

Sewage sludge was collected from the Dayton Municipal Wastewater Treatment Plant in Dayton, Ohio. Dayton sewage sludge contains both industrial and domestic influents and will be called "Dayton sludge". Dayton sewage treatment includes primary settling, anaerobic digestion, conditioning by the addition of lime, and dewatering in drying beds. Air dried Dayton sludge was collected in April, 1976, from drying beds, stored in plastic garbage cans, and was homogenized in the laboratory.

Application of Sewage Sludge to Soil

Miamian silt loam was mixed with perlite in a ratio of 5:2 (v/v). This soil-perlite mixture herein will be referred to as soil, and was

used in all potting experiments. Dayton sludge application to soil was calculated using the formula recommended by the Ohio Agricultural Research and Development Center (Grooms, 1975)[1]. Soil treatments were: the maximum (D. Max); one half the maximum (D/2); one fifth the maximum (D/5); one tenth the maximum (D/10) allowable amount calculated by this formula. Control Miamian soil (Con) had no Dayton sludge applied to it.

Following each harvest, treatments D/5 and D/10 received increments of Dayton sludge so that the soil in which the fifth crop grown in the D/5 treatment accumulated the same amount of sludge that was initially applied to the D. Max treatment. Similarly, treatment D/10 by the fifth crop equalled the initial application of D/2 treatment (Table 5).

Plant Growth

Seeds of sugar beet plants (Beta vulgaris, L. cv. USH 20), obtained from the Farmers and Marketers Sugar Beet Association, Saginaw, Michigan, were germinated in vermiculite moistened with deionized water. Two to four days following emergence, seedlings were washed free of vermiculite with deionized water and transplanted into 4-1/2 X 4-1/2 in plastic pots containing 600 cc of soil or sludge-amended soil. A layer of perlite, approximately 1 cm thick, was spread on top of soils to reduce the amount of water evaporation. Plants were placed in a controlled environmental chamber (Sherer, Model CEL 25-7 HL) programmed to provide a 12 hr light period at 25°C and a 12 hr dark period at 17°C. The chamber was illuminated by six fluorescent lamps (Westinghouse F48T12 CW/SHO) and two 25-watt incandescent lamps (General Electric). The

[1] See Appendix B.

Table 5. Mode of application of Dayton sludge (dry weight) to Miamian silt loam in experimental design in grams per pot and tons per acre.

Crop	Amount of sludge added	Con	D/10	D/5	D/2	D. Max
1	g/pot*	0	5.46	10.90	27.30	54.60
	t/a	0	2.51	5.02	12.55	25.10
2	g/pot	0	5.46	10.90	0	0
	t/a	0	2.51	5.02	0	0
3	g/pot	0	5.46	10.90	0	0
	t/a	0	2.51	5.02	0	0
4	g/pot	0	5.46	10.90	0	0
	t/a	0	2.51	5.02	0	0
5	g/pot	0	5.46	10.90	0	0
	t/a	0	2.51	5.02	0	0
Total added for 5 crops	g/pot	0	27.30	54.60	27.30	54.60
	t/a	0	12.55	25.10	12.55	25.10

*See Appendix B.

chamber walls and top were covered with aluminum foil producing a light intensity of approximately 1,800 - 2,000 ft-c at the level of leaf surfaces. Five replications of each treatment were placed into the chamber in a randomized block design. After the first week, pots were thinned to two plants per pot, and subsequently every two weeks, pots were rearranged in the chamber.

Prior to planting the first crop, all soils were fertilized according to the recommendations of the Ohio State University Soil Testing Laboratory. The amount of fertilizer added was 5.3 millimoles N, 0.88 millimoles P_2O_5 and 2.6 millimoles K_2O per pot. This is equivalent to 20 lbs of 6-24-12 (N,P,K) per 1,000 ft^2. Plants were grown for 49-52 days after transplanting and were then harvested. Following the harvest of the first crop, soils of the same treatments were homogenized, soil samples collected, soils put back into pots, and crop 2 planted. For treatments D/5 and D/10, the appropriate amount of Dayton sludge was added to the soil and the soil again homogenized following collection of soil samples. This procedure was followed for five croppings.

Plants were watered every other day with deionized water and once a week given 50 ml of half-strength Hoaglands nutrient solution (pH 4.5) minus $MnCl_2$, $ZnCl_2$, and $CuCl_2$. Iron was given by adding 1 ml of a standard 0.09 M $FeCl_3 \cdot 6H_2O$ solution per litre of half-strength Hoaglands solution. On the recommendation of Dr. Lee Sommers of Purdue University, Department of Agronomy (personal communication, 1976), nitrogen concentration of the nutrient solution was increased by 63%, by addition of 2.5 ml of 1 M NH_4NO_3 per litre of nutrient solution, to insure crop growth.

Plant Sample Preparation and Analysis

Plant samples were harvested by carefully removing soil from roots, divided into root and shoots (separated at the base of the crown), rinsed with deionized water, dried at 85°C and weighed to the nearest mg. Plants grown in the same pot were combined and ground in a Wiley Mill to pass through a 40-mesh screen and stored in 2-dram vials.

The plant powder was dried at 85°C for 2 hr, cooled in a dessicator and a 1-gram or smaller portion of the sample (weighed to the nearest 0.1 mg) was put into a porcelain crucible. Samples were dry ashed in a muffle furnace for 8 hr at 550°C, cooled and transferred to Folin-Wu tubes using a brush to retrieve all the ash. Ten ml of 3 N HCl was added to the tubes and samples were heated for 2 hr at 110°C in an aluminum block digester (Gallaher et al., 1975). The digest solution was allowed to cool, filtered, using Whatman No. 42 paper, into a 25-ml volumetric flask, and brought to volume with deionized water. The digest was transferred to acid-washed (HCl) plastic bottles and stored for analysis.

The digest was assayed directly by flame atomic absorption spectrophotometry (AAS) on a Perkin-Elmer, Model 603 spectrophotometer. Digest solutions containing low concentrations of cadmium were assayed using a graphite furnace (Perkin-Elmer, Model HGA 2100), otherwise, cadmium, zinc, copper, nickel and lead were assayed using flame AAS.

Soil and Sludge Analysis

Soil and sludge pH was determined in a 10 g soil:20 ml 0.01 M $CaCl_2$ slurry (Peech, 1965b), using a Corning Model 7 pH meter with a glass electrode accurate to within 0.05 pH units. Organic carbon content

of the soil and sludge was determined by the Walkley-Black method (Allison, 1965). Cation exchange capacity of samples was determined by sodium saturation[1] (Chapman, 1965). Exchange acidity was determined by the barium chloride-triethanolamine method (Peech, 1965a). Total exchangeable bases were calculated by subtracting exchange acidity from the cation exchange capacity. Total phosphorus was determined according to Sommers and Nelson (1972). Soil mechanical analysis was accomplished by the pipette method (Day, 1965). Total nitrogen was determined by both the Kjeldahl method (Bremner, 1965) and by the Dumas method using a Coleman Model 29 Nitrogen Analyzer (Tables 6 and 7, pages 37 and 38, respectively).

Preparation of soil and sludge samples for total cadmium, zinc, copper, nickel, lead, potassium, calcium, magnesium and sodium analysis by AAS was accomplished by dry ash digestion. Two-gram oven dried (105°C) soil samples and one-gram oven dried sludge samples were weighed in acid-washed (HCl) porcelain crucibles. Samples were then ignited at 550°C for 2-1/2 hr in a type 1500 Thermolyne Furnace, allowed to cool, and transferred to acid-washed Folin-Wu tubes. Twenty five ml of 3 N HCl were added to the sample tubes. Sample tubes were then placed into a preheated aluminum block heater on a Thermolyne type 2200 hot plate (Gallaher, 1975) and heated for 2 hr at 120°C. Samples were cooled, filtered through Whatman No. 42 paper and brought to volume in 50-ml volumetric flasks with deionized water, washing the residue with deionized water. Cadmium, zinc, copper, nickel, lead, magnesium and sodium concentrations were determined by flame AAS as above. Aliquots of

[1] See Appendix A.

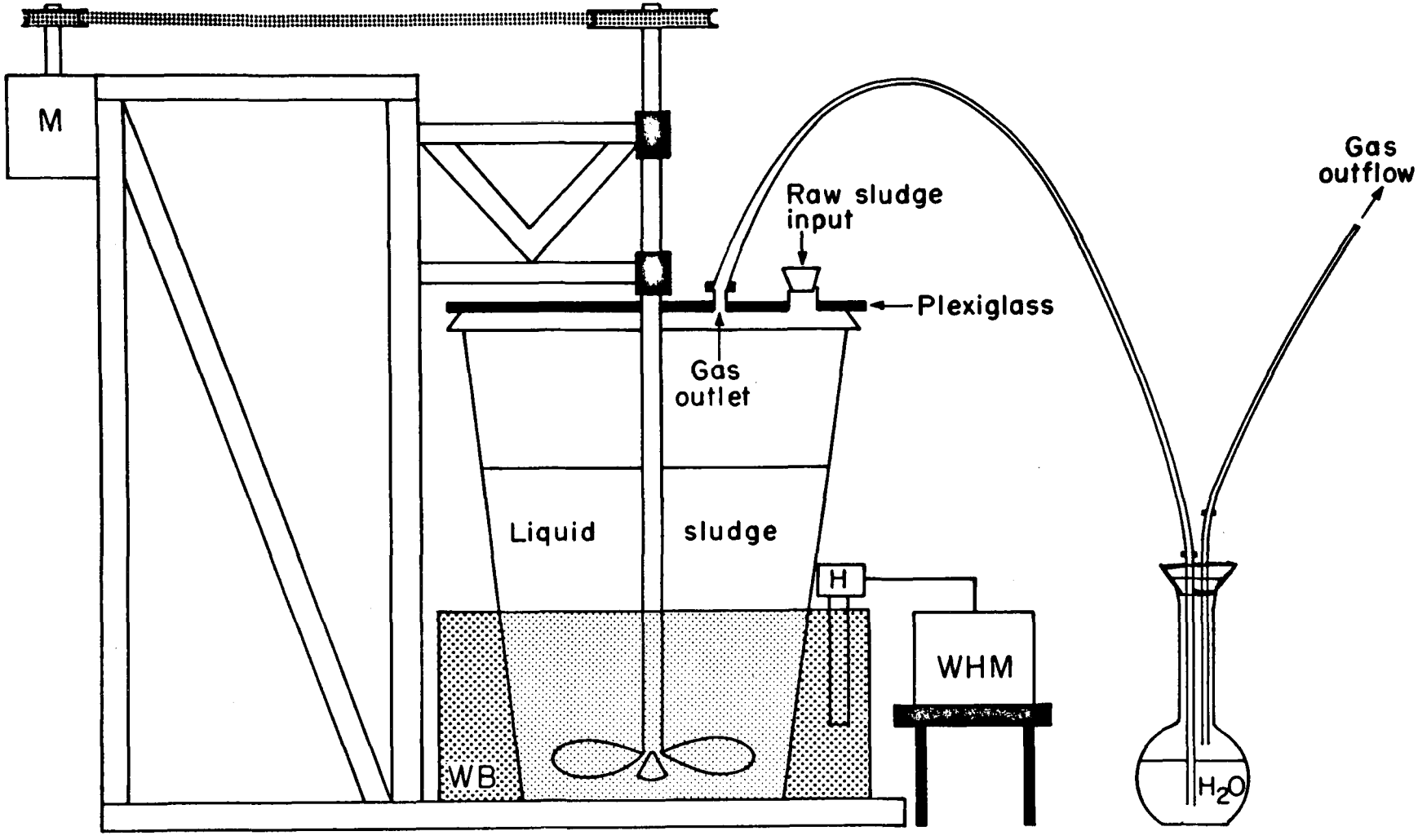
the digest solution were transferred to 25-ml volumetric flasks, the flasks brought up to volume with either 0.5% LiCl or 0.5% $SrNO_3$ solutions, and the concentrations of potassium and calcium, respectively, were determined by flame AAS (Tables 6 and 7, pages 37 and 38, respectively).

Extractable cadmium, zinc, cooper, nickel and lead in soil and sludge-amended soils were determined using a 0.005 M diethylenetriamine-pentaacetic acid (DTPA), 0.01 M $CaCl_2$ and 0.1 M triethanolamine (TEA) solution at pH 7.3 (HCl) as the extractant (Lindsay and Norvell, 1969). Ten grams of air dried sample was combined with 20 ml of DTPA extracting solution in 50-ml polycarbonate tubes (Type, Oak Ridge). Parafilm was placed over the tops of tubes and then caps were attached firmly to prevent leakage. The tubes were placed horizontally in a reciprocal shaker (Precision Instruments Co.) and shaken for one hr at approximately 280 oscillations per minute. Samples were then centrifuged at 3000 X gravity (Servall, Model RC-2) at 24°C for 20 min, and the supernatant filtered through Whatman No. 42 paper into polyethylene vials. The filtrate was analyzed directly for cadmium, zinc, copper, nickel and lead by flame AAS as above.

Production of Anaerobically Digested ^{109}Cd-labeled Sludge

Production of anaerobically digested ^{109}Cd-labeled sewage sludge was accomplished using a laboratory designed anaerobic digester (Fig. 1). Into a 62-gal plastic garbage can was added 32.2 l of secondary digested sludge obtained from the anaerobic digester at the Dayton Municipal Wastewater Treatment Plant, Dayton, Ohio. To this was added 13.7 l of raw sludge, collected from the bottom of settling beds, in small

Fig. 1. Design of the anaerobic digester used for the production of ^{109}Cd-labeled sewage sludge.



M - 3/4 horse-power motor
WB - Water bath
WHM - Water bath heater and circulator motor
H - Water heater and circulator

increments over a 24-day period. Four days after initiating digestion, 2 mCi of ^{109}Cd (2.43 mCi/mg Cd, New England Nuclear) in 500 ml 0.1 N HCl was added to the digester in two equal portions.

Digestion of the sewage sludge was maintained for 26 days at 33°C. The pH of the ^{109}Cd-labeled sludge was determined using pHydrion paper (pH range 6.0-8.0 in 0.2 increments) and was 7.4 for completely digested ^{109}Cd-labeled sludge. Percent volatile residue of the digested ^{109}Cd-labeled sludge was 47.2% ± 2.4% (S.E.) (dry weight basis) and was determined according to the United States Environmental Protection Agency (1974a) method. The ^{109}Cd-labeled sludge was 3.1% ± 0.03% (S.E.) solids determined by taking 10 ml of sludge, drying it at 105°C after it had been weighed, and comparing the weight of the dried sludge to the wet sludge. Cadmium concentration of Dayton sludge was determined to be 414 µg/g sludge on a dry weight basis. The resulting specific activity[1] of the ^{109}Cd-labeled sludge was 3.55 nCi/µg cadmium, on a dry weight basis.

Radioautography of Plants Grown on ^{109}Cd-labeled Sludge-Amended Soil

To 600 cc of Miamian silt loam was added: 368 ml (D/5); 184 ml (D/10) of ^{109}Cd-labeled sludge. The D/5 treatment was equivalent to one fifth the maximum and D/10 treatment was equivalent to one tenth the maximum amount of sludge allowable by the formula recommended by the Ohio Agricultural Research and Development Center (Grooms, 1975) discussed above. The ^{109}Cd-labeled sludge-amended soil was allowed to dry and fertilizer was added as in the section Plant Growth. One sugar beet plant per pot was grown, while all other conditions were similar to those in the section Plant Growth. Saran wrap was placed on top of

[1] See Appendix C.

pots in such a manner to allow plant growth and prevent any radioactive soil dust particles from becoming airborne and getting onto above ground portion of plants. Plants were harvested after 4, 5, 6, and 7 weeks of growth and shoots separated from roots. The shoots were rinsed with deionzed water, frozen between blotter paper with dry ice, lyophilized and placed onto Kodak Industrex AA X-Ray film for 5-6 weeks. Negatives, of sets of leaves being compared, were developed for the same length of time without changing the distance from the enlarger lens to the photographic paper and using the same f-stop for the enlarger lens. After exposure, all photographic paper was developed for two minutes in dektol, rinsed with water, fixed for six minutes and rinsed again with water for at least two hours.

Radioautographs of plants grown in ^{109}Cd-labeled sludge-amended soil were compared with control plants which were grown in soil to which non-labeled dry sludge was added at the D/5 treatment level as above.

RESULTS AND DISCUSSION

Analysis of Soil and Sludge

Miamian silt loam and Dayton sludge were analyzed for their chemical and physical properties, Tables 6 and 7, respectively. The percent of clay and pH of Miamian silt loam were found to be similar to the moderately fine-textured, high-lime glacial till Miamian silt loam of Montgomery County, Ohio (United States Department of Agriculture, 1976). Miamian silt loam is a calcareous soil containing a large concentration of calcium. It is also acidic, having a pH of 5.3, and does not exhibit the characteristic alkaline reaction of calcareous soils. Mechanical analysis of Miamian silt loam shows that soil separates add up to 122.5% (Table 6). Large variations in sample replicates for the mechanical analysis indicates that experimental error was responsible for the high sand and clay values obtained.

Dayton sewage sludge is an urban sewage sludge with industrial and domestic influents. It has a slightly low organic carbon content of about 21% (dry weight basis), the average being 25% (Miller, 1974). The large calcium concentration of Dayton sludge is due to addition of lime when conditioning the sludge after treatment. Concentrations of nitrogen, phosphorus and potassium in Dayton sludge are close to the median concentrations of these elements in sewage sludges from throughout the U.S.A. (Table 1). Concentrations of cadmium, copper, nickel, lead and zinc in Dayton sludge are also within the ranges found for these elements in sludges (Chaney, 1973), although their concentrations are much greater

Table 6. Chemical and physical properties of Miamian silt loam.

Analysis for:	Units	Mean	SE+	n	CV (%)++
Coarse sand*	%	5.6	0.3	8	15.0
Fine sand	%	28.3	2.7	8	27.0
Silt	%	47.8	1.3	8	7.7
Clay	%	40.8	3.1	8	21.5
Organic carbon	%	2.00	0.003	3	0.3
Cation exchange capacity	meq/100 g soil	22.49	0.65	10	9.1
Exchange acidity	meq/100 g soil	8.1	0.27	5	7.4
Exchangeable bases**	meq/100 g soil	14.39			
pH		5.35	0.03	3	1.0
Dumas-N	%	0.21		1	
Kjeldahl-N	%	0.20		1	
Total P	PPM	1,191	53	2	6.3
Total K	PPM	4,003	85	3	3.7
Total Ca	PPM	10,618	109	3	1.8
Total Mg	PPM	2,922	57	3	3.4
Total Na	PPM	1,042	190	3	31.6
Total Cd	PPM	0.7	0.01	4	0.3
Total Cu	PPM	9.95	0.04	3	0.1
Total Ni	PPM	19.3	0.3	3	2.7
Total Pb	PPM	24.1	0.8	6	8.1
Total Zn	PPM	73.4	1.3	6	4.3

+SE is standard error of the mean $(\frac{SD}{\sqrt{n}})$.

++CV is coefficient of variation $(\frac{\text{Standard Deviation}}{\text{Mean}})$.

*Classification of soil separates follow the International Society of Soil Science System (Day, 1965).

**Exchangeable bases determined by subtracting exchangeable acidity from cation exchange capacity.

Table 7. Chemical properties of Dayton sewage sludge (n = 3).

Analysis for:	Units	Mean	SE+	CV (%)++
Organic carbon	%	20.95	0.31	2.6
Cation exchange capacity	meq/100 g soil	28.43	0.95	5.8
Exchange acidity	meq/100 g soil	20.77	0.90	7.5
Exchangeable bases*	meq/100 g soil	7.66		
pH		6.34		
Dumas-N**	%	1.36		
Kjeldahl-N**	%	1.46		
Total P	PPM	15,540	580	6.5
Total K	PPM	1,278	15	2.0
Total Ca	PPM	95,025	1,045	1.9
Total Mg	PPM	9,506	79	1.4
Total Na	PPM	425	46	18.7
Total Cd	PPM	414	6.4	2.7
Total Cu	PPM	2,298	39	2.9
Total Ni	PPM	472	11	4.0
Total Pb	PPM	3,267	117	6.2
Total Zn	PPM	8,359	205	4.2
Cd:Zn ratio	%	5.0		

+SE is standard error of the mean $\left(\frac{SD}{\sqrt{n}}\right)$.

++ CV is coefficient of variation $\left(\frac{\text{Standard Deviation}}{\text{Mean}}\right)$.

*Exchangeable bases determined by subtracting exchange acidity from the cation exchange capacity.

** n = 1.

than the medians observed in Table 1. High heavy metal concentrations in Dayton sludge are characteristic of urban sludges and far exceed the maximum heavy metal concentrations in domestic sludges (Chaney, 1974). Comparison of heavy metal concentrations in Dayton sludge with those appropriate for agricultural land application (Table 2), reveals that with respect to heavy metals, addition of Dayton sludge to agricultural land would result in a poor benefit:risk ratio.

Growth of Sugar Beet Plants in Sludge-Amended Soil

Effects of addition of Dayton sludge to Miamian silt loam on sugar beet yield for five successive crops was investigated and the results are shown in Table 8 and Figure 2. Addition of Dayton sludge to Miamian silt loam significantly[1] affects growth of sugar beet plants in crop 1. Small sludge additions (D/10 and D/5) to soil does not significantly affect shoot yield (dry weight) while yields of sugar beet shoots from plants grown on soil treated with D/2 were significantly less than controls. Shoot yield of plants grown on the D. Max treated soil was significantly less than shoot yield from the D/2 treatment. Root yield of plants in crop 1 significantly decrease with increasing sludge application to soils.

Sugar beet shoots of crop 2 also showed a possible trend of decreased yield with increasing sludge application to soil. Roots of plants in crop 2 exhibited no such pattern. A possible trend is developed in crops 2 and 3 in which shoot yields of plants grown in control and D/10 soil treatments are equal and greater than shoot yields of plants grown in D/5, D/2 and D. Max treated soils (Table 8 and Fig. 2). Shoot yields of

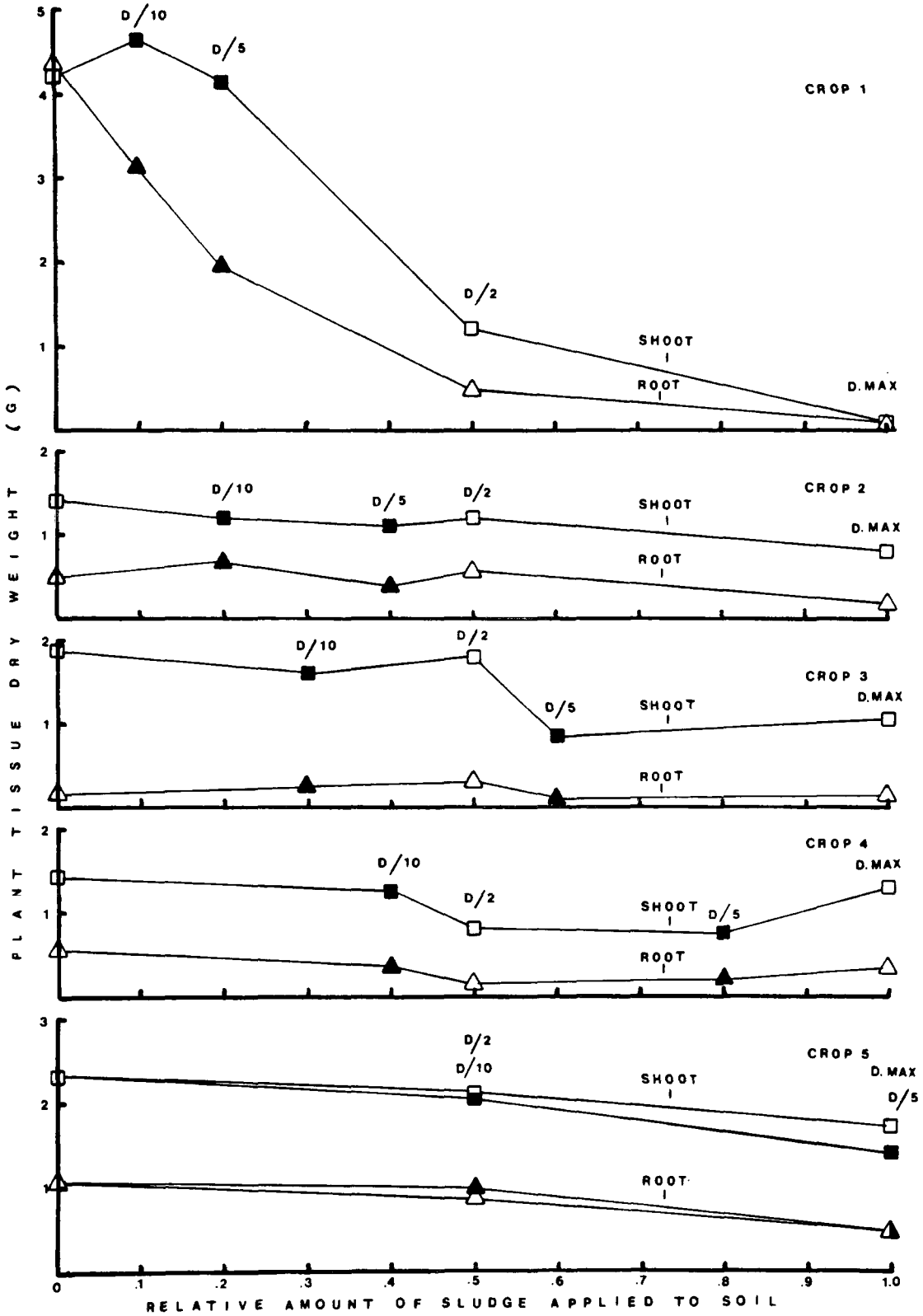
1. The word "significantly" will be used only in a statistical context, where $P \leq .05$ unless otherwise specified. Tests of significance only appears on tables.

Table 8. Means (n=5) of dry weight (g) of sugar beet plant tissue from five successive crops of sugar beets grown on Dayton sludge-amended Miamian silt loam.

		Tissue dry weight (g)				
		Treatment				
Plant part	Crop	Con	D/10	D/5	D/2	D. Max
Shoot	1	4.18^{a1*}	4.63^{a1+}	4.16^{a1}	1.20^{b1}	0.12^{c1}
	2	1.65^{a3}	1.45^{ab3}	1.08^{cd23}	1.22^{b2}	0.80^{d2}
	3	1.87^{a23}	1.60^{a23}	0.83^{b3}	1.80^{a2}	1.03^{b23}
	4	1.46^{a3}	1.25^{a3}	0.73^{b3}	0.79^{b3}	1.29^{a3}
	5	2.28^{a2}	2.05^{ab2}	1.39^{c2}	2.08^{ab2}	1.71^{bc4}
Root	1	4.33^{a1}	3.17^{b1+}	1.97^{c1}	0.47^{d1}	0.08^{d1}
	2	0.52^{ab23}	0.70^{a2}	0.34^{ab23}	0.62^{ab1}	0.20^{b1}
	3	0.15^{bc3}	0.23^{ab2}	0.11^{c3}	0.30^{a23}	0.14^{bc1}
	4	0.56^{a23}	0.36^{b2}	0.21^{b23}	0.17^{b3}	0.36^{b2}
	5	1.10^{a2}	0.94^{a2}	0.51^{b2}	0.89^{a4}	0.50^{b2}

*Means, within a set of treatments (rows) followed by the same letter (a-d) and of harvests (columns) followed by the same number (1-4) for shoots or roots, did not differ significantly at the 5% level according to Duncan's Multiple Range Test.
+Means of 4 replicates.

Fig. 2. Dry weight (g) of sugar beet shoots and roots from plants grown on Miamian silt loam treated with different modes of Dayton sludge addition for five consecutive crops. Soils which were treated with 1/10 the maximum (D/10) and 1/5 the maximum (D/5) amount of Dayton sludge received increments of 1/10 and 1/5 the maximum amount of sludge, respectively, following each harvest. These treatment modes are represented by (■) for shoots and (▲) for roots. Soils which received no Dayton sludge (Con), and soils to which 1/2 the maximum (D/2) and the maximum (D. Max) amount of Dayton sludge were applied initially, are represented by (□) for shoots and (△) for roots. The abcissa is in units relative to the maximum recommended amount of Dayton sludge ever to be added to Miamian silt loam.


CROP 1
D/10
D/5
D/2
SHOOT
ROOT
D.MAX
CROP 2
D/10
D/5
D/2
SHOOT
D.MAX
ROOT
CROP 3
D/10
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SHOOT
D.MAX
ROOT
CROP 4
D/10
D.MAX
D/2
SHOOT
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ROOT
CROP 5
D/2
D/10
SHOOT
D. MAX
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ROOT
PLANT TISSUE DRY WEIGHT (G)
0
.1
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.8
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1.0
RELATIVE AMOUNT OF SLUDGE APPLIED TO SOIL

crops grown on D/5 and D. Max treated soils did not differ significantly. In Crop 5 (Table 8), shootyields for control, D/10 and D/2 plants did not differ significantly while shootyields for D/5 and D. Max were significantly reduced compared to controls.

Shoot yield of control plants was always equal to or greater than shoot yield of plants grown on sludge-amended soils (Table 8). Root yield of control plants was also equal to or greater than root yield of plants grown on sludge-amended soils for all crops except crop 3. In crop 3, control root yield was significantly less than root yield of plants grown in D/2 treated soil (Table 2). During the third crop, the environmental chamber continually overheated to above 32°C. This constant overheating may have directly or indirectly (e.g., dessication of soil) stressed plants resulting in the small root yields obtained for all crop 3 plants except those grown in D/2 treated soils (Table 8).

Sabey and Hart (1975) observed that addition of low amounts of sewage sludge, 11.3 and 22.6 dry tons/acre (90, 2300, 250, 930 and 4800 ppm of Cd, Cu, Ni, Pb and Zn, respectively), to a sandy loam (C.E.C. of 5.2 meq/100 g soil) resulted in increased millet yields. Hyde (1976) reported increased corn and forage yields when sludge (40, 730, 270, 1000 and 4700 ppm of Cd, Cu, Ni, Pb and Zn, respectively) was applied to alkaline silt clay and clay loam soils at 3.3, 6.4 and 11.8 dry tons/acre.

Effects of applying an urban sludge containing 30, 539, 360, 200 and 3200 ppm of Cd, Cu, Ni, Pb and Zn, respectively, to Rideau clay (pH 5.6, C.E.C. of 18.7 meq/100 g soil) on yields of consecutive lettuce crops was investigated by Gaynor and Halstead (1976). They observed that application of 3.3 kg (50% moisture) sludge to 41 kg soil reduced lettuce yield for the first lettuce crop. Yields for consecutive crops did not

differ from those of chemically treated plots. They suggested that high salt concentrations in the soil due to sludge additions, may have been a factor in yield reduction of the first crop since consecutive croppings produced yields comparable to fertilized treatments. Jones et al. (1973) reported that initial salt concentrations either inhibited growth or were toxic to soybean plants grown on sludge-amended soil. Gaynor and Halstead (1976) report that other studies have also indicated high salt concentrations of sludge may retard or inhibit plant growth. In crop 1 of the present study (Table 8), reduction of sugar beet shootyields of plants grown in D/2 and D. Max treated soils and root yields for all sludge-amended soils, may have been in part due to a greater salt content of the soils treated with sludge.

In the present study, increased water retention with increasing amounts of sludge added to soils was observed. In soils treated with sludge, increased water retention may have decreased soil aeration, and thus contributed to inhibiting nutrient uptake thereby decreasing plant root and shoot yield. Yields of sugar beet shoots and roots from crop 5 are equal for D/10 and D/2 treated soils, and are significantly greater than shoots and roots from soils treated with D/5 and D. Max, which in turn are equal (Table 8). Comparison (t-tests) of crop 5 plants grown in D/10 and and D/5 treated soils with crop 1 plants grown in D/2 and D. Max treated soils, respectively, show that crop 5 yields are greater than those of crop 1 for shoots and roots. This data suggests that the amount of Dayton sludge and mode of application to Miamian silt loam affects sugar beet yield.

Yields of sugar beet shoots and roots from crop 1 of controls, D/10 and D/5 treatments were significantly greater than those of similar treatments from crops 2 through 5 (Table 8). Enhanced growth of crop 1 control, D/10 and D/5 plants was most likely the result of greater

availability of plant nutrients in crop 1 soils. Greater nutrient availability was due to fertilizing soils only prior to crop 1 with nitrogen, phosphorus and potassium, as recommended by the Ohio State University Soils Testing Laboratory. Fertilizing of soils only prior to crop 1 was done to optimize conditions for plant growth. In subsequent crops, no fertilizer was added to soils in order to investigate whether nutrients in sludge become available to plants, and to compare growth of crop 1 with that of subsequent crops. Depletion of available nutrients from control, D/10 and D/5 treated soils by the first crop resulted in consecutive crops not having a soil environment as favorable for growth as crop 1. Since root and shoot yields for crops 2-5 were less than crop 1 for the D/10 and D/5 treated soils, this may suggest that incremental addition of Dayton sludge does not replace available nutrients which are removed from the soil by crop 1. Also, since yields of roots and shoots of plants grown in D/10 and D/5 treated soils for crops 2-5 were never greater than control yields from respective crops (Table 8), this may suggest that Dayton sludge added in increments is not of any nutritive value to sugar beet crops grown on Miamian silt loam.

For roots and shoots of plants growing in D. Max treated soil, there is increased yields with successive crops (Table 8). No such trend is displayed by plants growing in D/2 treated soils. The possible trend exhibited by D. Max plants may result from release of plant nutrients from sludge organic matter as a consequence of microbial processes. Another factor which may be involved in influencing this trend is the salt concentration of the sludge-amended soils. With successive crops, salt concentrations of sludge-amended soil would decrease due to percolation of salt through the soil with each addition of water, and uptake and

removal of salts from soils by each successive crop. Regardless of the mechanism for increased crop growth with successive crops for D. Max shoots and roots, plants grown in sludge-amended soils had yields which were equal to or less than those of control plants of similar crops (Table 8).

Cadmium Accumulation in Sugar Beet Plants Grown in Sludge-Amended Soil

Shoots

Cadmium concentrations in sugar beet shoots from plants grown in any treatment of Dayton sludge-amended soil were not significantly different from controls except in one instance (Table 9). The cadmium concentration in sugar beet shoots of plants grown in the D/10 treatment of crop 5 accumulated significantly greater cadmium than did shoots of control, D/2 and D. Max plants. The greater cadmium accumulation by D/10 plant shoots of crop 5 cannot readily be explained, although may have been a result of experimental error.

Roots

Cadmium concentration in sugar beet roots grown in Dayton sludge-amended Miamian silt loam was always greater than shoots, as demonstrated by the ratio of root:shoot cadmium concentration (Table 10). In all control plants except for crop 2, a similar relationship between root and shoot cadmium concentration was apparent.

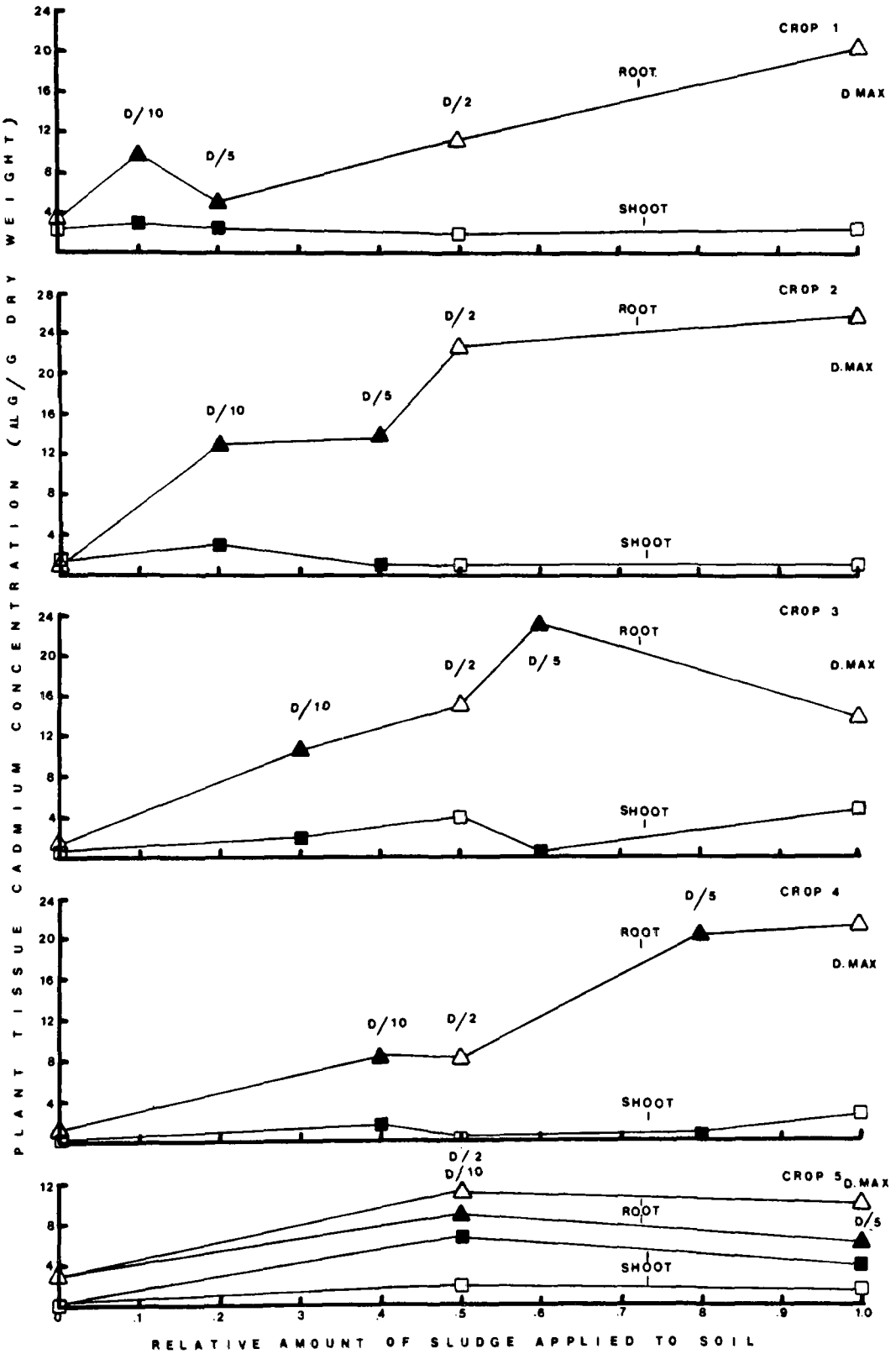
Preferential cadmium accumulation in various plant tissues have been demonstrated, and the tissue of preferential accumulation has been shown to be species specific. John et al. (1972a) showed that root cadmium concentrations of oat plants were greater than cadmium concentrations

Table 9. Means (n=5) of cadmium concentration (μg/g dry weight) in sugar beet plant tissue from five successive crops of sugar beets grown on Dayton sludge-amended Miamian silt loam.

		Tissue cadmium concentration (μg/g dry weight)				
		Treatment				
Plant part	Crop	Con	D/10	D/5	D/2	D. Max
Shoot	1	2.36^{a1*}	2.89^{a1+}	2.59^{a12}	1.97^{a1}	2.66^{a1}
	2	1.46^{a12}	2.80^{a1}	1.11^{a12}	1.03^{a1}	0.94^{a1}
	3	0.85^{a23}	2.13^{a1}	0.73^{a1}	3.96^{a1}	4.94^{a1}
	4	0.16^{a3}	1.61^{a1}	0.50^{a1}	0.30^{a1}	2.73^{a1}
	5	0.26^{a3}	6.41^{b1}	3.75^{ab2}	1.72^{a1}	1.49^{a1}
Root	1.	3.42^{a1}	9.87^{a1+}	4.91^{a1}	11.61^{ab1}	20.50^{b1}
	2	1.41^{a1}	12.98^{ab1}	13.98^{bc23}	23.02^{bc2}	25.72^{c1}
	3	1.29^{a1}	10.36^{ab1}	23.54^{c3}	14.85^{bc12}	14.17^{bc1}
	4	1.41^{a1}	8.69^{a1}	20.60^{b23}	8.37^{a1}	21.68^{b1}
	5	2.74^{a1}	8.87^{b1}	6.05^{ab1}	10.76^{b1}	9.95^{b1}

*Means, within a set of treatments (rows) followed by the same letter (a-d) and of harvests (columns) followed by the same number (1-3) for shoots or roots, did not differ significantly at the 5% level according to Duncan's Multiple Range Test.
+Means of 4 replicates.

Fig. 3. Cadmium concentrations (μg/g dry weight) of sugar beet shoots and roots from plants grown on Miamian silt loam treated with different modes of Dayton sludge addition for five consecutive crops. Soils which were treated with 1/10 the maximum (D/10) and 1/5 the maximum (D/5) amount of sludge received increments of Dayton sludge equal to 1/10 the maximum and 1/5 the maximum amount of Dayton sludge, respectively, following each harvest. These treatment modes are represented by (■) for shoots and (▲) for roots. Soils which received no Dayton sludge (Con), and soils to which 1/2 the maximum (D/2) and the maximum (D. Max) amount of Dayton sludge were applied initially, are represented by (□) for shoots and (△) for roots. The abcissa is in units relative to the maximum recommended amount of Dayton sludge ever to be added to Miamian silt loam.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D/10
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SHOOT
D/2
D/10
CROP 5
D.MAX
ROOT
D/5
SHOOT
PLANT TISSUE CADMIUM CONCENTRATION (µG/G DRY WEIGHT)
RELATIVE AMOUNT OF SLUDGE APPLIED TO SOIL

Table 10. Root:shoot cadmium concentration for sugar beet plants grown in Dayton sludge-amended Miamian silt loam.

	Root:shoot cadmium concentration				
	Treatment				
Crop	Con	D/10	D/5	D/2	D. Max
1	1.45	3.42	1.90	6.56	7.71
2	0.97	4.64	12.59	22.35	27.36
3	1.52	4.86	32.25	3.75	2.87
4	8.81	5.40	41.20	27.90	7.94
5	10.54	1.38	1.61	6.25	7.35

in tops of plants grown in cadmium polluted soil (95 ppm). In soils to which cadmium salts have been applied, cadmium concentrations of clover (Williams and David, 1977), oat, soybean, timothy, alfalfa, corn and tomato (MacLean, 1976) plant roots were greater than the cadmium concentrations in plant tops. Lettuce (John, 1972a; MacLean, 1976), carrot tobacco, potato (Maclean, 1976) and radish (John, 1972a and 1972b; Haghiri, 1973) plant tops have been reported to accumulate greater cadmium concentrations than roots when plants were grown in cadmium-enriched soils. For soybeans grown in cadmium-enriched solution culture, Collins et al. (1976) reported that cadmium concentrations in roots > stems > leaves. Jarvis et al. (1976) reported that cadmium concentrations for 23 plant species grown in solution culture containing 0.01 ppm cadmium ranged from 2.6-21.1 ppm in shoots and 18.9-151.1 ppm in roots.

In the first crop (Table 9), cadmium concentrationsin roots of plants grown in D. Max treated soils were significantly greater than cadmium concentrations in roots of plants grown in control, D/10 and D/5 treated soils. Cadmium concentrations in roots of D/2 plants were intermediate between the two groups. In crops 2 and 3, control plant roots had significantly less cadmium concentrations than did roots of plants grown in D/5, D/2 and D. Max treated soils. In crop 4 cadmium concentrations in roots of D/10, D/2 and control plants are not significantly different. Root cadmium concentrations of D. Max and D/5 in crop 4 (which now has received 4/5 the maximum amount of sludge ever to be applied) are similar, and significantly greater than root cadmium concentrations of control, D/10 and D/2 plants (Table 9). In crops 3 and 4, what may be occurring is that cadmium is being made available to plants by addition of sludge to the D/5 treatment coupled with the possible release of cadmium due to

microbial and plant processes from sludge previously added.

Hinesly et al. (1977), in a study regarding factors affecting cadmium concentrations in corn leaf and grain tissues, observed that sludge-borne cadmium incorporated into soil immediately prior to planting was the major determinant of levels of cadmium in these tissues when compared to amounts of cadmium supplied in sludge in previous years. This suggests that the majority of potentially available sludge-borne cadmium is made available to crops which are planted immediately following addition of sludge to soil. A possible mechanism for immediate availability of sludge-borne heavy metals has been proposed by Hinesly et al. (1977). They suggest that organic constituents of sludge maintained, for a short period of time, heavy metals in forms available for absorption by plant roots, probably by chelation processes. Over long periods of time, metal availability would decrease by reversion if sludge additions were terminated.

Results of crop 5 indicate that treatments D/10, D/2 and D. Max produced significantly greater cadmium concentrations in roots than did controls (Table 9). Plant root cadmium concentration of the D/5 treatment did not differ from controls in crop 5 possibly due to a large degree of variability within sample replicates. Compared to controls, cadmium concentrations in sugar beet roots is increased regardless of the mode of sludge addition.

Root cadmium concentrations of plants grown in control, D/10 and D. Max treated soils did not change during the course of five crops. In both D/5 and D/2 treatments, root cadmium concentrations were different over five croppings, but no pattern was evident.

In Table 9, t-tests of plant shoot or root cadmium concentrations

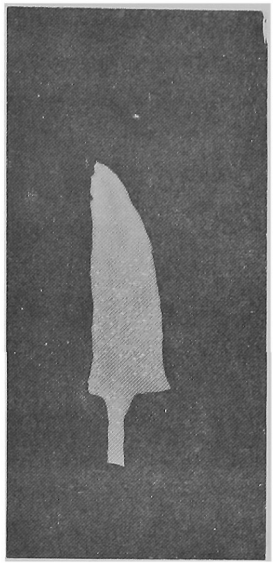
of crop 5 D/10 and D/5 treatments with crop 1 D/2 and D. Max treatments, respectively, showed that the concentration of cadmium differed significantly only in roots of plants from crop 5 D/5 and crop 1 D. Max treated soils. This suggests that the mode of Dayton sludge addition to Miamian silt loam does not effect sugar beet shoot cadmium concentration, but may affect root cadmium concentration.

Radioautography of shoots from sugar beet plants grown in ^{109}Cd-labeled Dayton sludge-amended Miamian silt loam was performed on 4, 5, 6 and 7 week old plants. Figure 4 shows leaves (5,3,1) of a 4-week old plant and leaves (9,7,5) of a 7-week old plant, the oldest leaf being leaf number 1. It appears that cadmium concentration in leaves increases with leaf age (Fig. 4). Chaney (1973) also reports that older leaves have greater cadmium concentrations than do younger leaves. Figure 5 shows the oldest leaf of 4, 5, 6 and 7-week old plants. Figure 5 shows a pattern of increasing cadmium concentration with plant age and cadmium concentrations in the leaf apex greater than the leaf base.

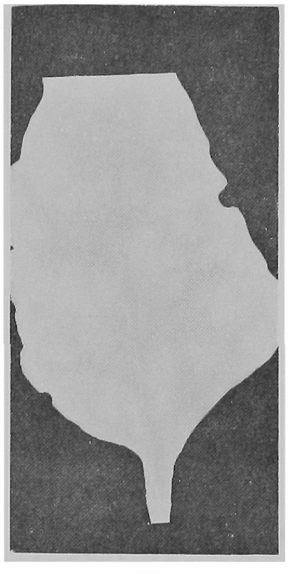
Cadmium concentration in sugar beet leaves appears in the order: midrib > principal lateral veins > leaf margin > mesophyll (Fig. 6). There appears to be an exclusion of cadmium in veins which branch off of the principal lateral veins (Fig. 6).

Sheriff and Meidner (1974) observed that water flowing along a leaf does so most easily through the vascular tissue, especially the midrib. Crowdy and Tanton (1970), using Pb-EDTA chelate to study the pathways of water movement in xylem and free space of wheat leaves, observed that the major volume of water in wheat leaves moved through the xylem, because lead was clearly visible in the xylem, where it was confined to the lumen

Fig. 4. Radioautographs of leaves 5,3 and 1 of a 4-week old plant (A-C, respectively) and 9,7 and 5 of a 7-week old plant (D-F, respectively) grown in ^{109}Cd-labeled sludge-amended Miamian silt loam. Amount of applied sludge was D/10. White areas indicate radioactivity. Within each group of three leaves, leaves accumulated higher concentrations of ^{109}Cd with increasing leaf age.



A



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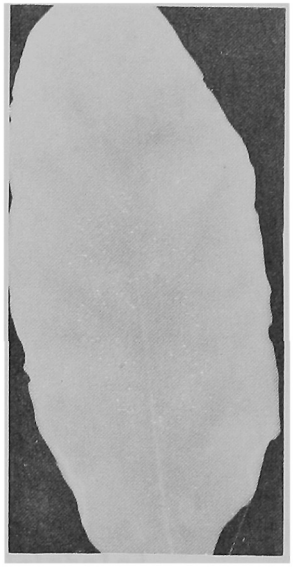

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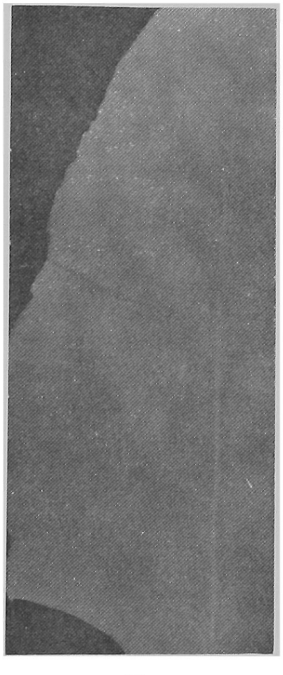
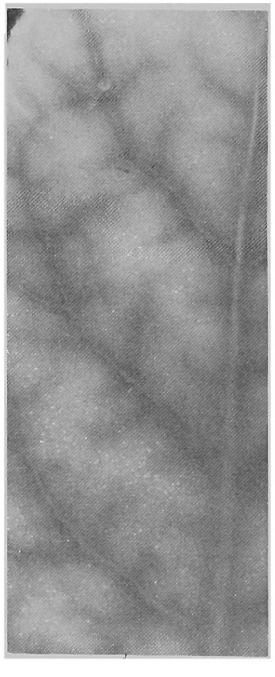


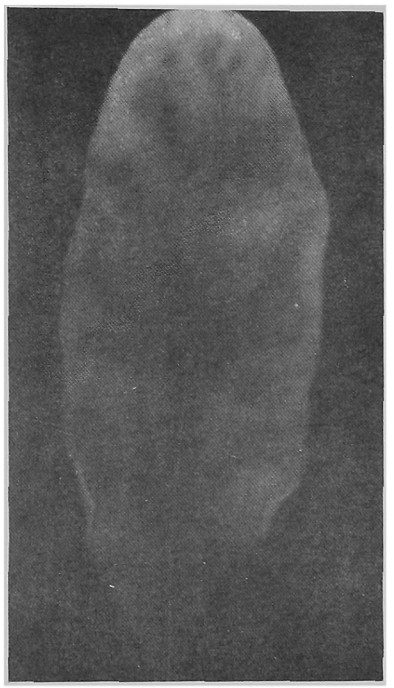
D

E

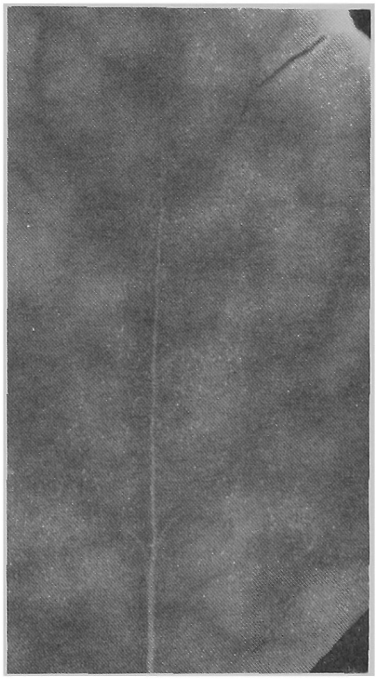


F

Fig. 5. Radioautographs of the oldest leaf of 4,5,6, and 7-week old plants (A-D, respectively) grown in ^{109}Cd-labeled sludge-amended Miamian silt loam. All plants were grown in soils to which was applied an amount of sludge equal to the D/10 treatment. Going from left to right, accumulation of higher concentrations of ^{109}Cd occurs with increasing plant age.



A



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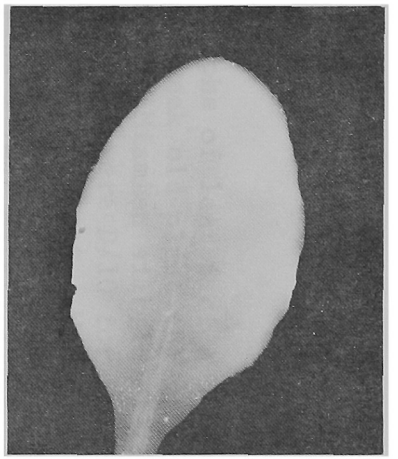
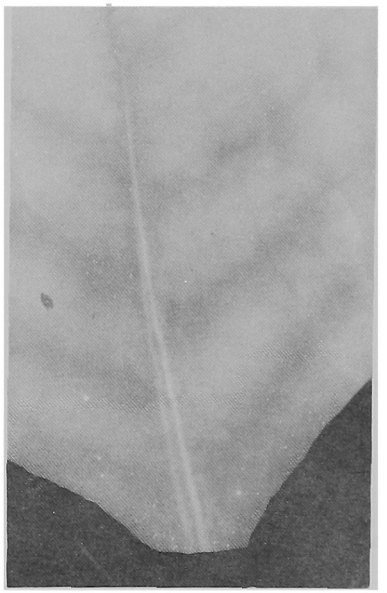
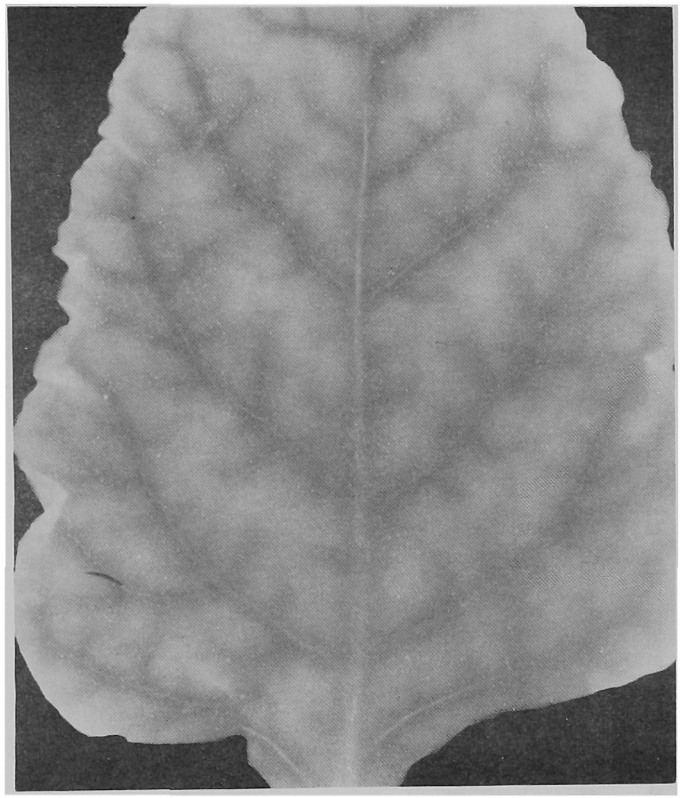


C

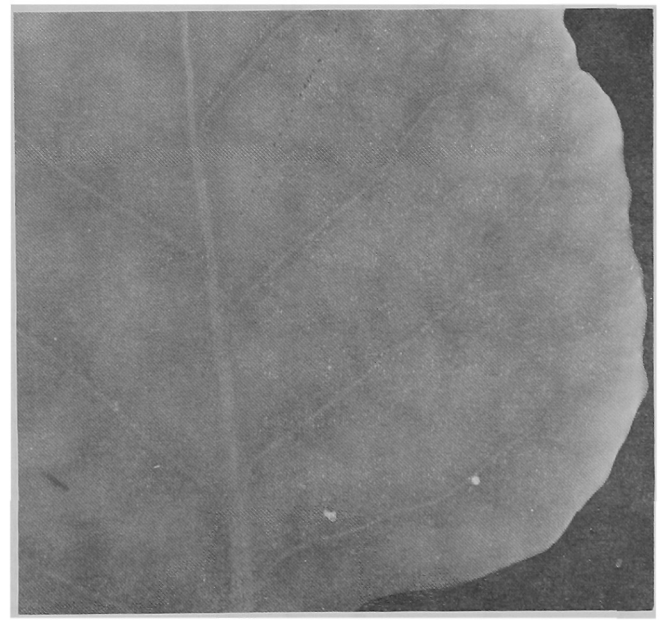


D

Fig. 6. Radioautographs of leaf 6 of a 7-week old plant (A) and leaf 5 of a 6-week old plant (B) of plants grown in ^{109}Cd-labeled sludge-amended Miamian silt loam. The amounts of sludge applied to soils were D/10 for the 7-week old plant and D/5 for the 6-week old plant. White areas indicate radioactivity. It appears that the ^{109}Cd accumulates in the midrib > principal lateral veins > leaf margin > mesophyll. It also appears that ^{109}Cd is excluded from the veins which branch off the principal lateral veins.



A



B

of vessels, being deposited on the inside of the walls. In the present study, cadmium in the transpiration stream acting analogously to lead, as reported above, may explain the large concentration of cadmium in the midrib and principal lateral veins.

After leaving xylem vessels, water moving through the mesophyll and epidermis travels almost exclusively through cell walls (free space) of living cells (Crowdy and Tanton, 1970). They observed that outside the xylem, lead deposits were confined to cell walls and were most dense in the middle lamella, coincident with the distribution of pectin.

The C-6 carboxyl groups of pectin (α-1,4-D-galacturonic acid polymers) in the middle lamella have been demonstrated to combine with calcium and magnesium (Salisbury and Ross, 1969), and may sequester cadmium from the transpiration stream in the free space of leaves. Sequestration of cadmium from the transpiration stream in the free space would explain the presence of cadmium in the mesophyll as well as explaining increasing cadmium concentrations in leaf tissue with increasing leaf age and increasing age of plants. This is because with increasing leaf age and increasing plant age, more water (which contains cadmium) passes through the leaf from which cadmium may be sequestered, resulting in the accumulation of greater concentrations of cadmium.

Crowdy and Tanton (1970) observed that entry of lead into wheat leaves was described by a diffusion model, and suggested that lead is translocated passively with water in the transpiration stream (xylem-free space system). Greater surface area:volume ratios in leaf marginal and leaf apex tissues compared to mesophyll tissue closer to the midrib and leaf base tissue, respectively, may result in a greater rate of transpirational water loss in these tissues. Assuming that water is transpired

from the leaf margin and apex at a greater rate than from tissue closer to the midrib and leaf base, respectively, and that cadmium acts analogous to lead in that it is translocated passively with water in the transpiration stream, then deposition of greater amounts of cadmium resulting from greater water transpiration would explain accumulations of cadmium in greater concentrations in the leaf margin and apex compared to tissue near the midrib and leaf base, respectively.

Cadmium Availability in Sludge-Amended Soil as Measured by DTPA-Extractable Soil Cadmium

Significant increases in the amount of DTPA-extractable cadmium was observed for all treatments in soils from prior to crop 1 to following crop 5 (Table 11 and Fig. 7). This suggests that incubation of Miamian silt loam and Dayton sludge-amended Miamian silt loam under sugar beet crop growth results in an increase in the amount of available (DTPA-extractable) cadmium. This may be due to a number of factors.

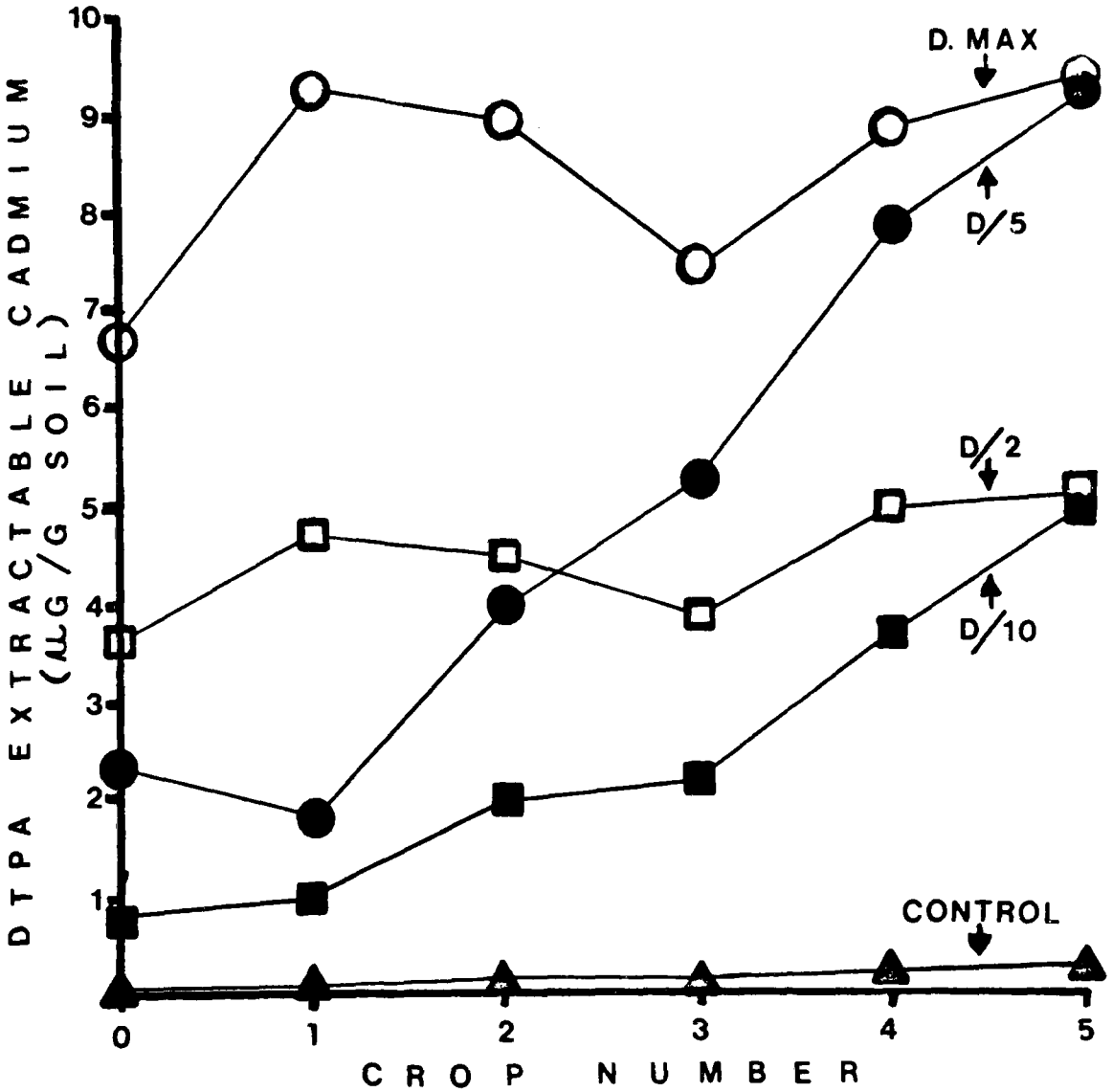
The first factor is the influence of plant roots on the rhizosphere. The release of H^+, CO_2 (which may be converted into carbonic acid in the soil solution), organic acids and amino acids (Quastel, 1963) into the rhizosphere by plant roots may lower pH of soil in the vicinity of roots. Subsequently, an increase in availability of heavy metals in soil may occur as discussed in the literature review. Jayman and Sivasubramanian (1975) observed that malic acid exudate from tea plant roots was capable of solubilizing iron and aluminum from insoluble iron and aluminum phosphates. Plant root mediated reduction of Fe^{3+} to Fe^{2+} by excretion of a reductant into the rhizosphere has been demonstrated (Rains, 1976). Nambiar (1976) reported that mucilage exudation from oat roots, in response

Table 11. DTPA-extractable cadmium (μg/g soil) from control and sludge-amended soils.

	DTPA-extractable cadmium (μg/g soil)				
	Treatment				
Crop	Con	D/10	D/5	D/2	D. Max
Before 1	0.09^{a1*}	0.78^{b1}	2.33^{c2}	3.63^{d1}	6.75^{e1}
After 1	0.09^{a1}	1.03^{b2}	1.84^{c1}	4.67^{d3}	9.31^{e4}
After 2	0.16^{a2}	2.00^{b3}	3.96^{c3}	4.49^{d2}	9.03^{e3}
After 3	0.14^{a3}	2.19^{b3}	5.31^{d4}	3.85^{c1}	7.48^{e3}
After 4	0.24^{a4}	3.69^{b4}	7.87^{d5}	4.96^{c4}	8.90^{c4}
After 5	0.27^{a5}	5.01^{b5}	9.30^{c6}	5.12^{b4}	9.38^{c4}

*Means, within a set of treatments (rows) followed by the same letter (a-e) and of harvests (columns) followed by the same number (1-6), did not differ significantly at the 5% level according to t-tests.

Fig. 7. DTPA-extractable cadmium from control soils and Dayton sludge-amended Miamian silt loam through five consecutive crops. Soil samples were taken prior to crop 1 (0 on the abscissa) and following crops 1-5.


DTPA EXTRACTABLE CADMIUM (µG/G SOIL)
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
CROP NUMBER
D. MAX
D/5
D/2
D/10
CONTROL

to water stress, may facilitate transfer of zinc to roots in dry soils. Excretion of amino and other acids by plant roots may increase the ability of the rhizosphere solution to bind metal ions in simple organic molecular-metallic complexes (Type-I complexes) which would facilitate uptake of heavy metals by plants (Zunino and Martin, 1977). Another factor which may increase the amount of available cadmium in incubated soils is the modification of soil by microorganisms as discussed in the literature review.

In control soils, the pH changed from 5.3 before crop 1 to 4.5 after crop 5 (Table 12). The decrease in control soil pH with successive crops may have been partially responsible for the observed increase in DTPA-extractable cadmium with successive crops in control soils (Table 11). Acidification of control soils may have been due to factors mentioned above (plant root modification of the rhizosphere or microbial modification of the soil) or the addition to soils of nutrient solution having a pH of 4.5. Hinesly et al. (1977) also observed that cropping soil (Blout silt loam) to corn for four years more than doubled the amount of extractable (0.1 N HCl) cadmium although he cautioned that 0.1 N HCl is a strong extractant for cadmium.

In soils treated with D/2 and D. Max, pH following crop 1 was higher than pH prior to crop 1 (Table 12). Regardless of the increase in pH from before to after crop 1, there was more extractable cadmium following crop 1 (Table 11) for the D/2 and D. Max treated soils. The greater amount of extractable cadmium following crop 1 may have been due to time of incubation of the sludge-amended soil.

After crop 1, consecutive crops lowered soil pH for D/2 and D. Max soils after crop 1 to crop 5, available cadmium did not greatly increase

Table 12. Soil pH* of control and Dayton sludge-amended Miamian silt loam treatments.

	Treatment				
Crop	Con	D/10	D/5	D/2	D. Max
Before 1	5.3	5.7	5.8	5.8	5.9
After 1	5.4	5.7	6.0	6.5	6.8
After 2	5.6	5.5	6.0	6.2	6.6
After 3	5.5	5.6	5.6	5.7	6.1
After 4	4.6	5.65	5.9	5.7	5.9
After 5	4.55	5.65	5.8	5.8	6.0

*0.01 M $CaCl_2$ (1:2 0.01 M $CaCl_2$).

between crops. In D/2 and D. Max treated soils, 70% and 97%, respectively, of the cadmium made available by incubation of the sludge-amended soils for five crops, was made available during the first six-week crop.

Extractable cadmium from D/10 and D/5 treated soils increased with successive crops grown on the soil (Table 11 and Fig. 7). This seems reasonable due to additions of increments of sludge immediately prior to each of the five crops. Table 13 gives data concerning the relative amount of added extractable soil cadmium for D/10 and D/5 treated soils following each of the five harvests compared to the amount of extractable soil cadmium for D/2 and D. Max treated soils, respectively. Table 13 shows that the relative amount of extractable cadmium from D/10 and D/5 treated soils closely approximates the predicted amount of extractable soil cadmium from D/2 and D. Max treated soils, respectively. If cadmium made available from sludge-amended soils was directly related to incubation time of the sludge-amended soils, then the relative amount of soil extractable cadmium from D/10 and D/5 treated soils after crop 5 compared to soil extractable cadmium of D/2 and D. Max soils, respectively, would not be close to 1, but considerably less. But, if the majority of potentially available cadmium in the sludge-amended soil is immediately made available (during the initial 7-week period) following a sludge application to soil, providing that the rate of reversion of cadmium is small, then the relative amount of soil extractable cadmium from D/10 and D/5 treated soils after crop 5 would equal the amount of extractable soil cadmium of D/2 and D. Max treated soils, respectively.

Zunino and Martin (1977) suggest that simple organo-metallic complexes (Type-I compexes) in soils may be of greater importance in determining the availability of heavy metals in soils. In the Dayton sludge added to Miamian silt loam, the complex polymerized organic

Table 13. Amount of DTPA-extractable soil cadmium of the D/10 and D/5 treated soils relative to the amount of DTPA-extractable soil cadmium following crop 5 of the D/2 and D. Max treated soils, respectively.

Crop	Theoretical	Actual	
		D/10 D/2	D/5 D. Max
Before 1	.20	.21	.35
After 1	.20	.22	.20
After 2	.40	.45	.44
After 3	.60	.57	.71
After 4	.80	.74	.88
After 5	1.00	.98	.99

molecules with numerous electron-donor functional groups (Type-II complexants) present may be completely saturated with cations, resulting in Type-III complexes, due to the large concentrations of cations (especially calcium) in Dayton sludge. Therefore, a large proportion of heavy metals in Dayton sludge may form Type-I complexes, due to the preponderance of simple organic acids in sludge as a consequence of anaerobic digestion (United States Environmental Protection Agency, 1974b). These Type-I complexes would most likely be the first molecules utilized by soil microorganisms as an energy source.

Microbial utilization of these simple soluble organo-metallic complexes in the soil solution would possibly result in the soil becoming increasingly acidic. Acidification of the soil would result from substitution of H^+ ions from cation exchange sites on simple organic acids by heavy metal ions in the soil solution. Consequently, the formation of organo-metallic Type-I complexes and the increase in H^+ ion concentration in the soil solution would result. Either decreased heavy metal availability (as a result of the removal of Type-I complexes from the soil solution and accumulation of heavy metals by microorganisms) or increased heavy metal availability (as a consequence of soil acidification) in the soil would result depending on which of the factors mentioned above was dominant.

In the present study, during the first 7-week incubation (crop 1), pH of D/2 and D. Max treated soils increased (Table 12) concomitant with the release of the majority of cadmium that was made available during the course of the five crops (Table 11 and Fig. 7). Silviera and Sommers (1977) reported a similar pattern of changes in metal extractability with time from sludge-amended soil. They reported that of the amount of

DTPA-extractable soil cadmium obtained after incubation of Fox silt amended with sludge from Kokomo, Indiana for 44 weeks, 88% was obtained after only 6 weeks of incubation. For Fox silt amended with sludge from Logansport, Indiana, they observed that of the amount of extractable soil cadmium obtained after incubation for 44 weeks, 69% was obtained after 6 weeks of incubation.

No change in pH of D/10 and D/5 treated soils was observed through five crops (Table 12). In Table 11, the amount of extractable soil cadmium from D/10 and D/5 treated soils after crop 5 was compared to the amount of extractable soil cadmium from D/2 and D. Max treated soils after crop 1, respectively, using t-tests. The comparisons showed that the amount of extractable soil cadmium from D/5 treated soil did not differ significantly than that from D. Max treated soil after crop 1. But, the amount of extractable soil cadmium from D/10 treated soil after crop 5 differed significantly than that from D/2 treated soil after crop 1. Therefore, whether the mode of Dayton sludge addition to Miamian silt loam affects the amount of extractable soil cadmium cannot be ascertained from the data.

Correlation of DTPA-Extractable Soil Heavy Metals with Yield and Heavy Metal Uptake

Stepwise multiple regression analysis between DTPA-extractable soil heavy metals and yield (dry weight) or heavy metal content of sugar beet plant tissue from crop 1 plants was performed to determine the possible effects of soil extractable heavy metals on crop yield and tissue heavy metal concentration (Table 14). Regression analysis was reported just for crop 1. Addition of nitrogen, phosphorus and potassium to only

Table 14. Correlation coefficients[+], r, from stepwise multiple regression analysis between DTPA-extractable soil heavy metals and yields (dry weight) or heavy metal content (μg/g dry weight) of plant parts from crop 1 plants grown in control and sludge-amended soils.

Plant yield and heavy metal Content	Correlation coefficients, r, of DTPA soil extractable heavy metals				
	Zn	Cu	Ni	Cd	Pb
Shoots					
Dry weight			-.934***	(.660***)++	
Zn				.890***	
Cu	.945***				(-.591***)++
Ni	.561**				
Cd					
Pb	.763***				
Roots					
Dry weight		-.889***			
Zn				.904***	
Cu	.927***				(-.480**)++
Ni	.546**				
Cd	.640***				
Pb	.801***				

+Correlation coefficients are given only for those values which are significant at the .01 level.

*** $P \leq .001$.
** $P \leq .01$.
++Partial correlation coefficients.

crop 1 soils prior to planting resulted in optimal growth conditions for the first crop. Soil for subsequent crops did not have additions of these plant nutrients prior to planting and crops were most likely under stress as a result of a nutrient deficiency. This is illustrated by control plants in crop 1 having significantly greater yields than control plants of subsequent crops (Table 8).

Plant shoot and root dry weight may have been adversely affected by available soil nickel and copper, respectively (Table 14). Phytotoxicity of nickel and copper has been well documented (Gaynor and Halstead, 1976). They also reported that a great reduction in sugar beet and lettuce yields as a result of adding zinc-, copper- and nickel-contaminated sludges to soil was observed.

Page (1974) reported that poor growth of oat and potato plants grown on sludge-amended soils was attributed to nickel toxicity. Generally, copper is twice as toxic (Chaney, 1973), and nickel is four times (Grooms, 1975) or eight times (Chaney, 1973) as toxic to plants as zinc. In the present study, soil available cadmium has a significant positive partial correlation with shoot dry weight (Table 14). Therefore, statistically holding available nickel in sludge-amended soil constant, it appears that soil available cadmium may enhance sugar beet growth.

Table 14 shows that plant tissue zinc concentration is significantly correlated with available soil cadmium. This may suggest a possible synergistic affect of available soil cadmium on zinc uptake by sugar beet. Plant shoot copper, nickel and lead and root copper, cadmium, nickel and lead concentrations have significant positive correlations with soil available zinc (Table 14).

MacLean (1976) reported that 5-50 ppm soil-applied zinc increased the cadmium concentration in soybean shoots. In cadmium-enriched solution cultures, antagonistic effects on the uptake of cadmium by potassium, aluminum (John, 1976), manganese, zinc (Jarvis et al., 1976) and iron (Iwai et al., 1975) have been reported. Francis and Rush (1974) reported that increasing zinc concentrations in cadmium-enriched nutrient solutions decreased root cadmium concentrations, but significantly increased cadmium concentrations of leaves and stems of bush beans. Cunningham et al. (1975), in a study concerning the effects of various levels of metal-enriched sludge-amended soil on metal uptake by plants found significant positive correlations between zinc concentrations in the sludge-amended soil and copper and nickel concentrations in tops of corn and rye plants. The results obtained in the present study may suggest synergistic affects of extractable soil zinc on the uptake of copper, nickel and lead by sugar beet shoots and copper, nickel, cadmium and lead by sugar beet roots. A significant negative partial correlation between soil available lead and plant tissue copper concentrations was also observed. This may suggest an antagonistic effect of soil available lead on the uptake of copper by sugar beet plants.

SUMMARY AND CONCLUSIONS

Dayton sludge should not be utilized as a fertilizer or soil conditioner on Miamian silt loam when sugar beet crops are to be grown. Yield decrement, possibly due to heavy metal toxicities (especially nickel and copper) or to excessive salt concentrations (Jones et al., 1973) may occur with additions of sewage sludge equal to or greater than one half the maximum amount permissible as calculated by the formula which is recommended by the O.A.R.D.C. (Grooms, 1975). Therefore, this formula should be modified to lower the maximum permissible amounts of sludge-borne heavy metals allowed to be added to agricultural soils, and/or take into consideration the affect of sludge-borne salts on crops.

Addition of equal amounts of Dayton sludge to Miamian silt loam by different modes (either initially or in increments) does not affect the cadmium concentrations of successive crops of sugar beets. Large variations in cadmium concentrations of replicate samples, resulting from experimental error, may have obscured differences in cadmium concentrations of successive crops with respect to mode of sludge addition. Successive croppings of sugar beets after initial applications of the entire amount of Dayton sludge to Miamian silt loam did not affect the cadmium concentrations in crops. Because the availability of sludge-borne cadmium to sugar beet plants, as measured by tissue cadmium concentration, does not change over five croppings, it appears that sugar beet crops grown following termination of Dayton sludge addition to Miamian silt

loam will not increase in cadmium concentrations compared to the last crop which was planted prior to termination of sludge additions. Incremental additions of Dayton sludge to Miamian silt loam reduces yield decrement compared to inital additions of an equal amount of sludge. Since the mode of Dayton sludge addition to Miamian silt loam does not affect the cadmium concentration of sugar beets, greater yield decrement resulting from initial sludge additions to soil may in part be a consequence of the large salt concentrations in the sludge-amended soil.

Cadmium accumulation occurs in sugar beet roots > shoots. Increases in root cadmium concentration with increasing amounts of Dayton sludge applied to Miamian silt loam may be due to greater nonmetabolic sequestering of cadmium by roots from soils as soil cadmium concentration increases. Nonmetabolic sequestering of cadmium by sugar beet roots from soils may also explain the greater cadmium concentration of roots compared to shoots. Observations that cadmium is transported to shoots of plants indicates that cadmium must enter cells at some point. Cadmium concentration in sugar beet shoots increases with increasing plant age and increasing age of plant leaf. This pattern of accumulation suggests that cadmium is relatively immobile in sugar beet shoots.

In sugar beet leaves, cadmium accumulates in the midrib > principal lateral veins > leaf margin > mesophyll. Cadmium concentrations in the leaf margin and apex is greater than that in the tissue closer to the midrib and leaf base. Cadmium being translocated from roots to shoots in the transpiration stream (xylem-free space system) would account for the large concentrations of cadmium in the midrib and principal lateral veins. Sequestration of cadmium ions from the transpiration stream in the leaf free space primarily by pectin would explain the presence of cadmium in the

mesophyll, as well as explaining increasing cadmium concentrations in leaf tissue with increasing leaf age and increasing age of plants. Assuming that cadmium is translocated passively with water in the transpiration stream, and that the leaf margin and apex have a greater rate of transpirational water loss than does leaf tissue closer to the midrib and the leaf base, accumulation of cadmium in greater concentrations is expected in the leaf margin and apex than in the tissue closer to the midrib and leaf base.

Addition of Dayton sludge to Miamian silt loam increases the amount of total and DTPA-extractable soil cadmium. Miamian silt loam treated with increasing amounts of Dayton sludge results in the increase of extractable soil cadmium under successive sugar beet crops. The majority of potentially available sludge-borne cadmium is available to crops which are planted immediately following sludge application to soil which suggests that the majority of potentially available sludge-borne cadmium may be complexed with simple organic compounds, possibly in Type-I complexes. The large concentration of cations in sludge (especially calcium in Dayton sludge) saturating Type-II complexants thereby forming Type-III complexes together with the large concentration of organic acids in anaerobically digested sewage sludge also lends evidence towards the possibility that a large amount of the sludge-borne cadmium exists in Type-I complexes.

A positive correlation between extractable soil cadmium and zinc uptake by sugar beet plants grown on Dayton sludge-amended Miamian silt loam was observed. This suggests that a possible synergistic affect of available soil cadmium on the uptake of zinc may exist. Dayton sludge has a Cd;Zn ratio of 5.0%. Chaney (1973) suggests that sludges having a Cd;Zn ratio of greater than 0.5% should not be applied to agricultural land. For sludges which have a Cd:Zn ratio of less than or equal to 0.5%,

the possible synergistic affect of available soil cadmium on zinc uptake by plants may have little consequence.

Positive correlations between extractable soil zinc and the uptake of copper, nickel and lead by shoots and copper, nickel, cadmium and lead by roots of sugar beet plants grown on Dayton sludge-amended Miamian silt loam were observed. These positive correlations suggest synergistic affects of available soil zinc on the accumulation of copper, nickel, cadmium and lead by plant tissue. If zinc does synergistically affect uptake of copper and nickel by sugar beets grown on sludge-amended soils, then increasing zinc concentrations in sludge may increase the toxic affects of copper and nickel by increasing plant uptake of these elements. If this is the case, modification of the formula recommended by the O.A.R.D.C. should be made to take into consideration the affects of high zinc concentrations on the bioaccumulation of copper and nickel.

Elucidation of the mechanism by which heavy metals in sludge-amended soils are made available to plants will involve determination of how heavy metals are complexed in the sludge-soil mixture. If it were known how heavy metals are complexed in sludge-amended soils, ways to reduce their uptake by plants and their subsequent entrance into the human food chain could be devised. Further investigations may reveal that the behavior of heavy metals is dependent upon the particular sludge, soil and plant being investigated.

Generally, sludges containing small amounts of heavy metals are less of a potential hazard to the human food chain when applied to agricultural soils than sludge containing large amounts of heavy metals. Once heavy metals are incorporated into sludge, there is no practical method presently available which can remove them. Therefore, the reduction of heavy metal

concentrations in industrial sludges (such that they may be safely utilized as agricultural soil amendments) should ultimately occur at the source of heavy metal pollution. Decreasing the amount of heavy metal pollution from its source would entail the passage and enforcement of legislation which allows safe levels of heavy metals in industrial effluents.

APPENDIX A

Determination of Cation Exchange Capacity by Sodium Saturation[1]

Four grams of oven dried (105°C) Miamian silt loam ($\leq$ 2mm) was placed into a 50-ml polycarbonate centrifuge tube (Type, Oak Ridge). Next, 33 ml of 1.0 N NaOAc (pH 8.2) was added to the centrifuge tube, the tube shaken on a mechanical shaker at approximately 280 oscillations min^{-1}, and centrifuged at 3,000 X gravity for 10 min. The supernatant was decanted and the extraction was repeated three more times. Samples were then washed with three 33-ml portions of 99.9% 2-propanol (low in sodium, J.T. Baker Chemical Co.).

Using the shaking and centrifugation procedure above, the samples were extracted with three 33-ml portions of 1.0 N NH_4OAc (pH 7.0), decanting each washing into a 100-ml volumetric flask. Extracting solutions in the 50-ml volumetric flasks were diluted to the mark with 1.0 N NH_4OAc, a 0.1-ml aliquot was taken, put into a 25-ml volumetric flask, diluted to the mark with deionized water, and sodium concentration was determined by flame AAS.

Calculation of Cation Exchange Capacity of Miamian Silt Loam

Given: Sodium adsorbed by Miamian silt loam[2] (μg Na/g soil) = 5,171

Atomic weight of sodium = 23 g $mole^{-1}$

[1] Chapman, 1965.

[2] Calculated from the amount of sodium in the NH_4OAc desorption solution determined by AAS.

1 mole sodium = 1 equivalent (eq)

Calculation of milliequivalents per 100 g soil:

$$\frac{5{,}171\ \mu g\ Na}{g\ soil} = \frac{517{,}100\ \mu g\ Na}{100\ g\ soil}$$

$$\frac{517{,}100\ \mu g\ Na/100\ g\ soil}{23 \times 10^{6}\ \mu g\ Na/mole} = 2.25 \times 10^{-2}\ mole\ Na/100\ g\ soil$$

$$\frac{2.25 \times 10^{-2}\ mole\ Na}{100\ g\ soil} = \frac{2.25 \times 10^{-2}\ eq}{100\ g\ soil}$$

$$\frac{2.25 \times 10^{-2}\ eq}{100\ g\ soil} \times \frac{10^{3}\ meq}{eq} = \frac{22.5\ meq}{100\ g\ soil}$$

APPENDIX B

Calculation of the Maximum Permissible Amount of Dayton Sludge Addition Ever Allowed to be Applied to Miamian Silt Loam

Given: Cation exchange capacity of Miamian silt loam is 22.5 meq/100 g soil.

Metal concentration of Dayton sludge in ppm:
Zn - 8,359
Cu - 2,298
Ni - 472

Maximum amount of Dayton sludge ever allowed on Miamian silt loam:

$$\frac{22.5 \times 16,350}{8,359 + 2(2,298) + 4(472) - 200} = 25.12 \text{ tons/acre}$$

Conversion factor for tons/acre to grams/centimeter^2:

$$\text{Ton} \times \frac{2,000 \text{ lbs}}{\text{Ton}} \times \frac{453.6 \text{ g}}{\text{lb}} = 907,200 \text{ g}$$

$$\text{Acre} \times \frac{4,047 \text{ m}^2}{\text{Acre}} \times \frac{10,000 \text{ cm}^2}{\text{m}^2} = 40,470,000 \text{ cm}^2$$

$$\frac{T}{A} = \frac{907,200 \text{ g}}{40,470,000 \text{ cm}^2} = 0.02242 \text{ g/cm}^2$$

Therefore: $25.12 \text{ T/A} \times 0.02242 = 0.563 \text{ g/cm}^2$

Given: Surface area of pot used in experiments is 96.8 cm^2

Maximum amount of Dayton sludge allowed per pot of Miamian silt loam

$0.563 \text{ g/cm}^2 \times 96.8 \text{ cm}^2\text{/pot} = 54.5$ g sludge/pot (dry weight basis)

APPENDIX C

Calculation of the Specific Activity of ^{109}Cd-Labelled Digested Sewage Sludge

Given: 45.9 l of liquid sludge which is 3.09% solids and having a specific gravity of 0.96.

Cadmium concentration in the sludge is 414 μg Cd/g dry sludge.

Total amount of activity added to the sludge was 2.002 mCi ^{109}Cd having a specific activity of 2.43 mCi/mg.

Specific activity of digested sludge.*

$$45.9 \times \frac{0.0309 \text{ g dry sludge}}{\text{g liquid sludge}} \times 0.96 = 1.361 \text{ kg dry sludge}$$

$$1.361 \text{ kg dry sludge} \times \frac{414 \text{ mg Cd}}{\text{kg dry sludge}} = 563.5 \text{ mg Cd}$$

$$\frac{2.002 \text{ mCi}}{563.5 \text{ mg Cd}} = \frac{3.55 \text{ μCi}}{\text{mg Cd}} = \frac{3.55 \text{ nCi}}{\text{μg Cd}}$$

*The addition of 2.002 mCi ^{109}Cd (specific activity = 2.43 mCi/mg Cd) added less than 1 μg Cd/g sludge (dry weight).

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Compounds of Zinc and Copper in Sewage Sludge Determined by Electron Spectroscopy[1]

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ABSTRACT

Electron Spectroscopy for Chemical Analysis (ESCA) was used to provide information concerning the chemical bonding of zinc and copper present in the sludge obtained from the Dayton, Ohio sewage treatment plant. The binding energy of the Zn $2p_{3/2}$ electron in sludge when placed under a vacuum of 10^{-7} Torr was 1023.8 ± 0.2 eV and the binding energy of the Cu $2p_{3/2}$ electron in the sludge under similar conditions was 937.2 ± 0.3 eV. These energies are characteristic of both Zn and Cu being in the positive two oxidation state. The electronegativity of the ligand attached to either of these metals was found to be 3.5 ± 0.1 on the Pauling scale. This indicates that Zn and Cu exist in similar compounds, that the ligand binding site is oxygen, and that the possible compounds include carbonate, carboxylate, phosphate, nitrate, silicate, and/or oxide. ESCA, when used in conjunction with other methods, offers excellent potential for more specifically identifying the chemical form of metal in sludge or sludge mixtures.

Additional Index Words: molecule identification, compound identification, chemistry, analytical chemistry, electron spectroscopy for chemical analysis (ESCA), photoelectron spectroscopy.

It has been shown that sewage sludge can be of value in conditioning some soils (1). In highly industrialized cities, however, the sludge is likely to contain significant amounts of heavy metals. Among these are Cu and Zn which are essential for life and others such as Cd which can be deleterious (8, 14). It has also been observed that an excess of any element can cause negative and even toxic effects to plants. Of major concern then is the possibility that these metals could be concentrated by plants and animals and eventually consumed by man. If it were known how metals are chemically combined in sludge and the forms transported from sludge to plants grown on sludge-covered fields, one might devise ways to reduce or even eliminate plant uptake. Also, such data would enable one to more accurately predict the migration of metals in soil profiles.

Generally, only the amount of metal in a sludge, plant or, soil sample is measured; however, there are times when it is important to know the chemical form of a metal. Whether a metal in a particular compound is dangerous or toxic depends on its availability to plants. For example, elemental mercury is considerably less toxic than methyl mercury. Likewise, hexavalent Cr is highly toxic, while if it present as trivalent Cr it is much less harmful.

One might expect that the growing of plants in sludge containing 10,000 ppm Zn would inhibit or even prevent growth. However, if the Zn is bound in a form that makes it unavailable to the plants, no adverse effect will result. Thus, the danger level of the amount added to the soil should be given for the compound, not simply the amount of metal added.

Studies of metal content of environmental samples so far have most often determined only the amount of metal (14), and only guesses have been made concerning the actual chemistry of the metal (9, 11). Some of the studies involved determining the extractability of metals using chelating agents and acids (10, 12), which only provide information on chemical forms in broad terms, rather than identifying the metal compounds specifically. Attempts to determine stability constants (15) are valuable but face the same problem of not uniquely determining the compound. In solution, these metals are expected to exist as simple or complex ions or uncharged molecules; however, the kind of chemical compound which controls the solubility in sludge or sludge-amended soils indicates the kind of chemical activity possible.

The basic question of how metals are complexed in environmental samples has been unanswered because of the unavailability of a method which can determine chemical identity of trace levels of a metal (2, 4). This paper will discuss the use of electron spectroscopy for chemical analysis (ESCA) as a tool to be used in conjunction with other techniques in providing information concerning the chemical compounds of Zn and Cu present in Dayton, Ohio sludge. Cadmium was also of interest but the concentration was too low to be observed by ESCA but should be detectable by mass spectroscopy. This latter possibility will be discussed later.

In this paper it will be shown that ESCA may be a valuable tool in determining the complexes formed by heavy metals in sewage sludge. From the ESCA spectra of Dayton sludge it was determined that Zn and Cu are complexes with an oxygen-containing ligand.

MATERIALS AND METHODS

Studies on Dayton sludge by atomic absorption showed a high concentration of many heavy metals. In particular concentrations of Zn, Cu, and Cd were 10,030 ± 163, 2,409 ± 19, and 414 ± 11 ppm, respectively. Quantitative information is not as accurate using ESCA. In general, ESCA is a qualitative rather than a quantitative tool although it does yield some quantitative information.

ESCA, sometimes called photoelectron spectroscopy, can provide molecular compositional information. In this technique (16), electrons are ejected with either X-rays or ultraviolet light and their kinetic energies (E_k) are measured with either electric or magnetic analyzers. By knowing the energy of the exciting radiation, $h\nu$, the electron binding energy (E_b) is obtained from the relationship $E_b = h\nu - E_k - \psi$, where ψ is an instrumental number depending on the work functions of the sample and spectrometer. If atoms are bonded with different ligands or in any way are subjected to different fields, the binding energy of the electrons is slightly different. Depending on the element and compound, the shift in binding energy is usually in the range of 0.1 to 10 eV. For many situations the resolution of present instruments is sufficient to give information on bonding and, thus, indicate the molecule involved. If the emerging electron suffers no collisions with other molecules before it is detected, its spread in energy will be dependent primarily on the inherent X-ray energy width and some spreading due to the instrument used. The resulting electron energy spread for

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presently available instrumentation at half maximum (FWHM-full width of half maximum) is of the order of 1 eV.

For the present study a modified AEI ES-100 Photoelectron Spectrometer was used. The photon source was Mg X-rays of energy 1253.6 eV. All measurements were made at a vacuum of about 10^{-7} Torr. It is possible that some compounds will sublime in vacuum and this is difficult to determine with present techniques. However analysis of samples using secondary ion mass spectroscopy may clarify this situation. Electrons have a mean free path in most materials which is short enough so that if they were produced more than two or three atomic layers in bulk, they will lose enough energy to be scattered out of the photoelectron peak.

Since the energy shifts are so small, calibration is quite important. Using a procedure developed earlier (3, 13) a compound with a known binding energy was mixed into the unknown sludge sample. The sludge sample was dried at room temperature and powered in an agate mortar to form a powder of similar particle size to that of the known compounds (zinc phosphide and copper iodide). The binding energies of several Zn compounds have been determined earlier (3, 13). Some shifts for Cu compounds have been measured (6) while others were determined in this study.

RESULTS AND DISCUSSION

The $Cu2p_{3/2}$ and $Zn2p_{3/2}$ ESCA photoelectron spectra are shown in Fig. 1-3. The data shown has had the background subtracted and has been smoothed (7). The lines through the data points are intended to guide the eye while the individual peak shapes have been constructed from shapes for the individual compounds indicated. Figure 1 shows the $Cu2p_{3/2}$ electron spectra for a mixture of CuI_2 and CuO. As can be seen, a shift of 2.0 eV exists between the peaks due to the two compounds.

Other mixtures of copper compounds produce the shifts listed in Table 1. The electron spectrum for the

Table 1—ESCA energy shifts for some Cu compounds

Compounds	Difference of $Cu2p_{3/2}$ electron energy between compounds
	eV
$CuI_2/CuCO_3$	1.5 ±0.3
CuI_2/CuO	2.0 ±0.2
CuF_2/CuI_2	3.0 ±0.2†
CuF_2/CuI_2	2.8 ±0.2†

† Two photoelectron peaks were observed for $Cu2p_{3/2}$ in CuF_2. We have chosen the lowest binding photopeak as reference. The doublet can be explained in terms of final states and localization of *d*-electrons. A number of final states are possible due to spin-spin or spin-orbit interaction of the unpaired *d*-electrons with the $2p_{3/2}$ hole created in the photoionization process (6). The difference in energy between the final states is a function of the delocalization of the unpaired Cu *d*-electron onto the ligand. For F the delocalization is small, therefore, the split is large; for CuCN the reverse is true.

$Cu2p_{3/2}$ lines of the mixture of sludge and copper iodide is shown in Fig. 2. A similar spectrum for the $Zn2p_{3/2}$ lines of the mixture of sludge and zinc phosphide is shown Fig. 3.

In mixing the standards into the sludge, care was taken to make the concentrations of the metals equal. The relative size of the standard and sample peaks for all samples analyzed is shown in Fig. 2 and 3. The sludge Zn peak was lower and the sludge Cu peak was higher than that of the standard. Since ESCA is surface sensitive, this suggests that Cu is more concentrated than Zn on the surface of the particle of the sludge. This parameter will be discussed in a later paper.

Using the data from Cothern et al. (3), the relative position of the peak in the $Zn2p_{3/2}$ electron spectrum for different ligands is shown in Fig. 4 as a function of energy.

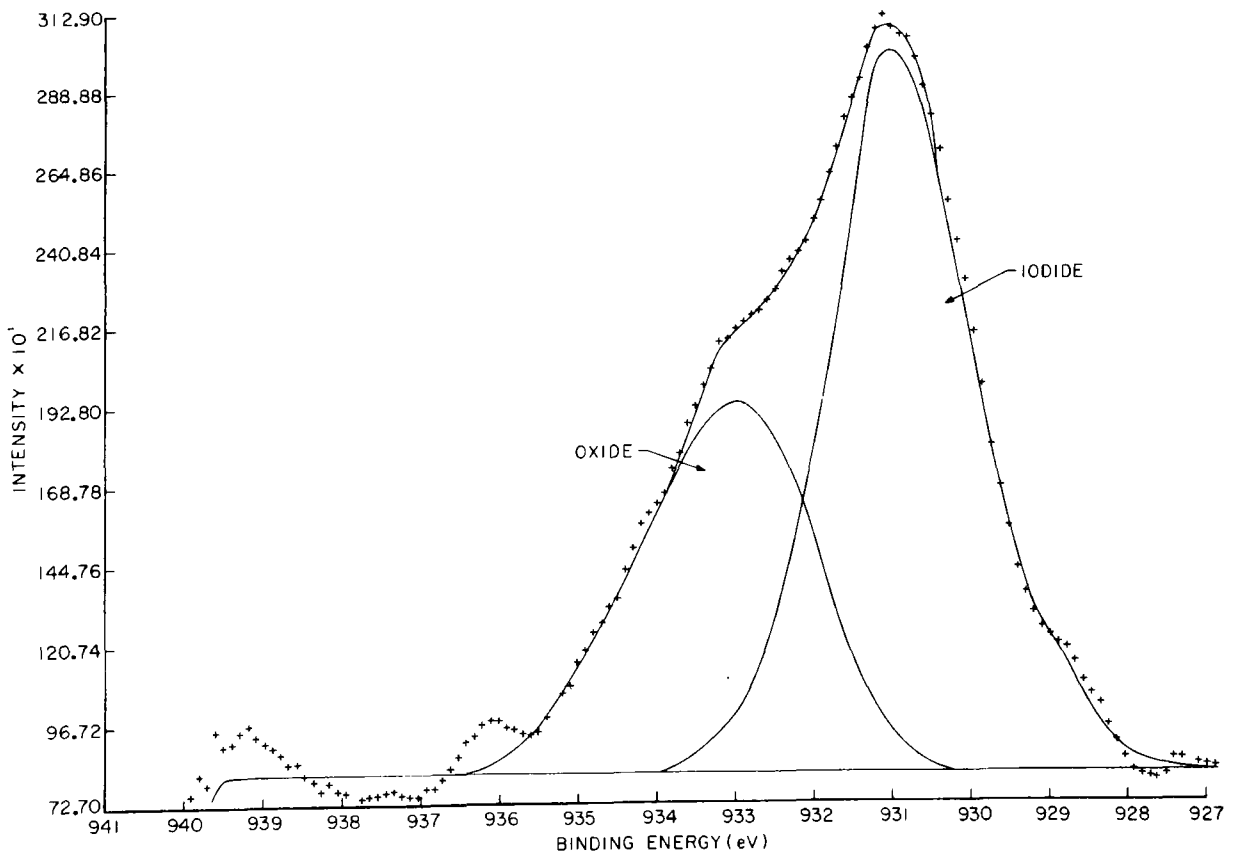

Fig. 1—Electron spectrum of $Cu2p_{3/2}$ electrons ejected from a mixture of CuI_2 and CuO. The energy shift was 2.0 ±0.2 eV. The solid curves were determined from single compound data and fitted to the two compound data shown.

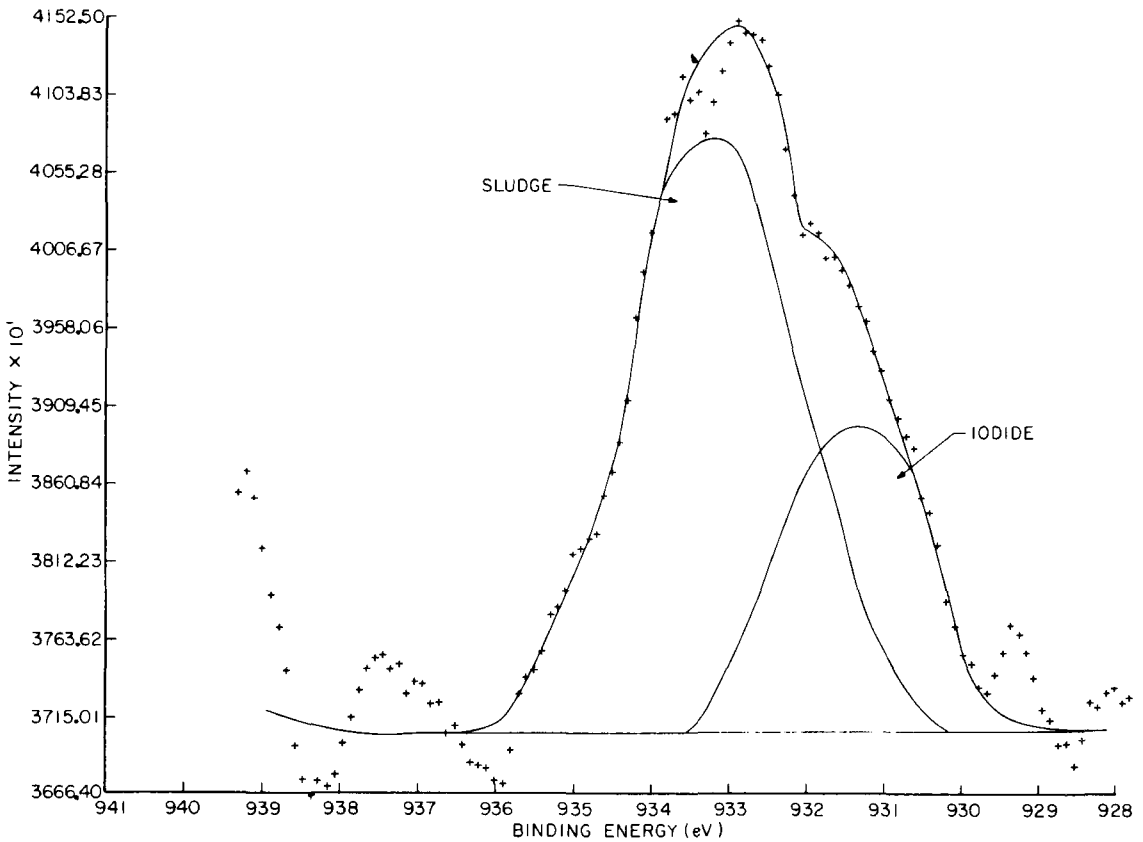

Fig. 2—Electron spectrum of $Cu2p_{3/2}$ electrons ejected from a mixture of sludge and CuI_2. The energy shift was 2.0 ± 0.2 eV. The solid curves were determined from single compound data and fitted to the two compound data shown.

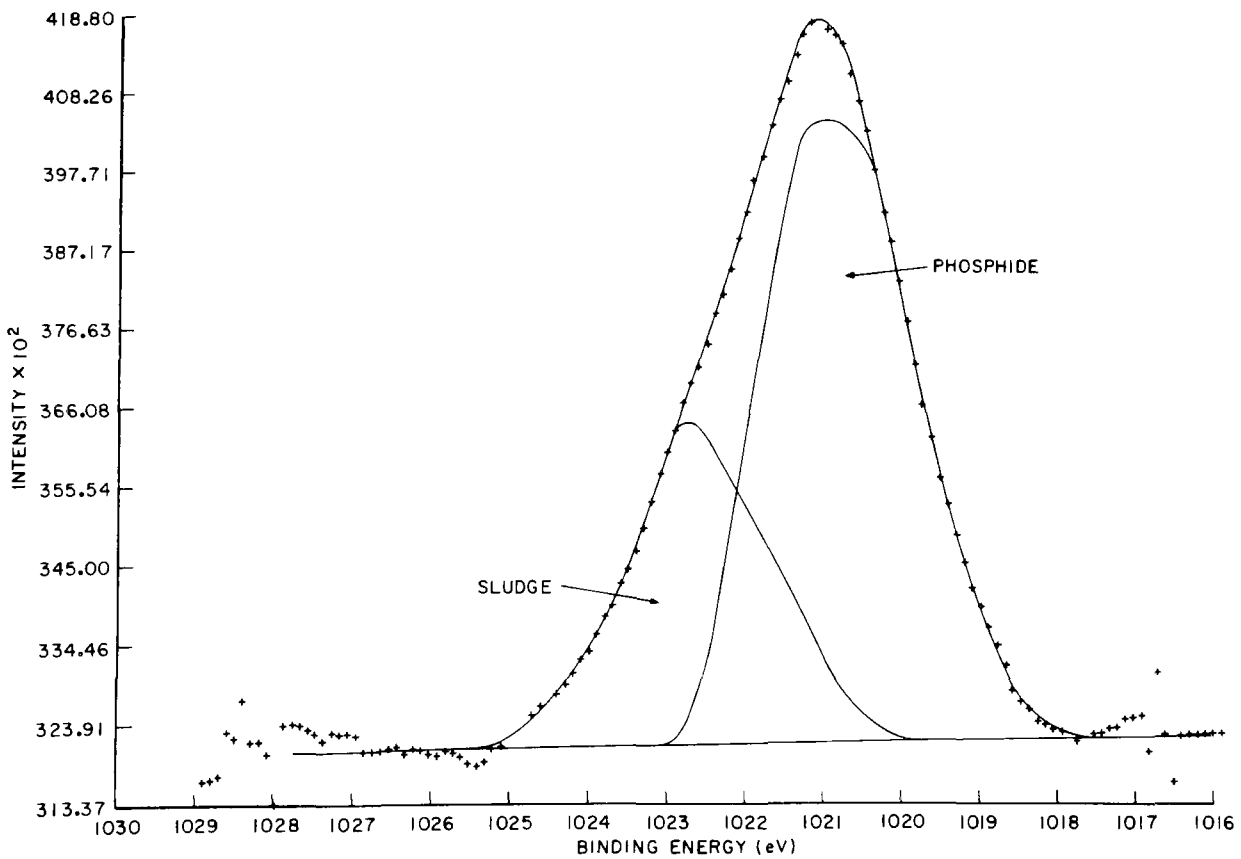

Fig. 3—Electron spectrum of $Zn2p_{3/2}$ electrons ejected from a mixture of sludge and Zn_2P_3. The energy shift was 1.7 ± 0.2 eV. The solid curves were determined from single compound data and fitted to the two compound data shown.

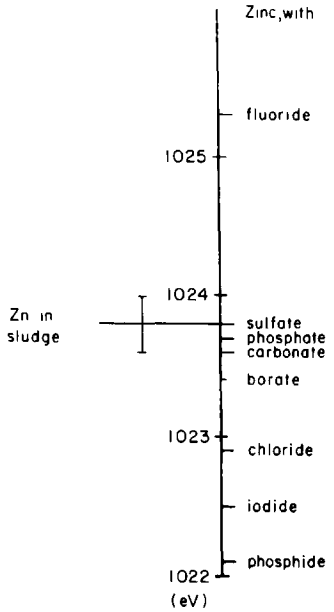

Fig. 4—Plot of $Zn2p_{3/2}$ electron energies for different ligands (Cothern et al., 3). Also shown is the value for Dayton, Ohio sludge. The vertical scale is electron binding energy.

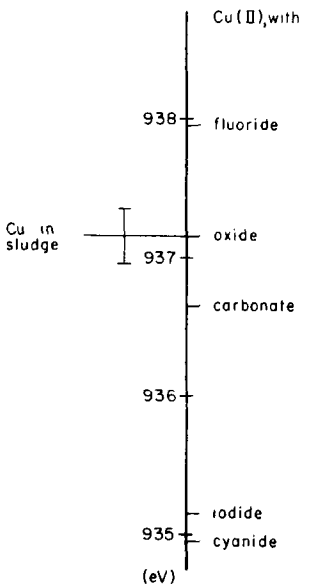

Fig. 5—Plot of $Cu2p_{3/2}$ electron energies for different ligands. Also shown is the value for Dayton, Ohio sludge. The vertical scale is electron binding energy.

Although the absolute position on the scale has an error of ± 0.4, the relative positions of the ligands is correct to ± 0.2 eV. Also indicated in Fig. 4 is the energy value for the $Zn2p_{3/2}$ line in sludge. From this plot it is deduced that the Zn in sludge is bound to a ligand with electronegativity of 3.5 ± 0.1 on the Pauling scale (3, 5, 13). It can be further observed that to achieve this high value, the ligand must contain oxygen, and could be an oxide, carbonate, phosphate, nitrate, sulfate, or silicate. Previous analysis suggest an inorganic silicate (9) or an organic carboxylate (14) as possibilities. If the ligand is organic in nature, bidentate binding through a carboxylate is quite feasible. A similar plot for Cu is shown in Fig. 5. Interestingly the value for the electronegativity of the ligand of Cu in sludge is also 3.5 ± 0.15. Because of the similarity of the plots, the same conclusions as made for Zn also apply to Cu.

The sensitivity of ESCA is often quoted as one part in 10^3 to 10^4. For the present sample Cd is found to fall outside the range; therefore, ESCA may not be sensitive enough to detect Cd in sludge samples. Another technique involving mass spectroscopy is sensitive enough. Secondary ion mass spectroscopy (SIMS) is more sensitive (to one part in 10^6). It is hoped that this technique will yield information concerning the chemical compounds of Cd present in sludge. For comparison, the sensitivity limit for atomic absorption is in the range of one part in 10^6 – 10^9, but in that case only the total concentration is obtained.

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COMPARISON OF SAMPLE PREPARATION TECHNIQUES FOR ATOMIC ABSORPTION ANALYSIS OF SEWAGE SLUDGE AND SOIL

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ABSTRACT

Five techniques for preparing sludge and soil for atomic absorption determination of trace amounts of cadmium, lead, nickel, copper, and zinc were evaluated. Dry ashing, involving heating at 550°C then leaching with acids was found to be the best technique. It is the fastest and it results in the best precision and the most accurate assays.

COMPARISON OF SAMPLE PREPARATION TECHNIQUES FOR ATOMIC ABSORPTION ANALYSIS OF SEWAGE SLUDGE AND SOIL

By

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INTRODUCTION

At the present time, there is considerable interest in applying sewage sludge to agricultural lands. This practice would help solve the sludge disposal problem of cities and the sludge serves as a good low-grade fertilizer and soil conditioner. However, the rather high heavy metal content of some sludges presents a potential health problem. Considerable effort is being made to determine the amounts of heavy metals in sludge and soil and how much of and in what form these move into food crops or into surface and ground water. Atomic absorption spectroscopy was used in this study to determine heavy metal concentrations.

There is some controversy over what is the best sample preparation method for atomic absorption analysis of the "total" amounts of trace heavy metals in sewage sludge, soil and other biological materials. Most methods involve one of two processes, 1) dry ashing, which consists of heating samples to approximately 500°C followed by acid leaching, 2) wet ashing, which consists of digestion with HNO_3 and $HClO_4$ at approximately 120°C. Several comparison studies of the two methods, used to prepare plant materials for analysis, have been reported (1, 2). These studies show that comparable results are obtained with the two procedures. Castellano (1) found that the precision of the dry ashing method is significantly

(statistically) better. In addition, he found that the dry ashing was faster and he concluded that it was a better digestion procedure than wet ashing.

In the present study, three sample preparation methods for determining total heavy metal concentrations in soil and sludge were investigated: (1) dry ash digestion, (2) wet ash (HNO_3- $HClO_4$) digestion, (3) HNO_3 extraction. A fourth method, wet ashing with HNO_3 and HCl was used on one sludge and a fifth method, using total digestion with HF, was used on another sludge.

EXPERIMENTAL

Approximately 100 Kg of each of a local major soil type and two sludges from the Dayton, Ohio, areawere collected. The soil is Miami silt loam, an upland till soil. It was collected from the upper six inches of the "A" horizon. One sludge is from the city of Dayton waste water treatment plant which handles both domestic and industrial sewage. The other sludge is from Englewood, a Dayton suburb, and is almost completely domestic. The dried sludges were collected from sand drying beds. The soil and sludges were air dried, mixed, passed through a 2 mm screen, and oven-dried overnight at 110°C. The five digestion methods are described as follows:

Dry Ash Digestion

Two-gram samples of soil and one-gram samples of sludge were weighed and transferred into acid-washed porcelain crucibles. The samples were then ignited at 550°C for 2½ hrs. in a muffle furnace (1). They were then cooled and placed in acid-washed 50 ml Folin-Wu test tubes. Twenty-five mls of 3 N HCl were added to each tube, and the tubes were placed in holes in an aluminum block heater (3). The samples were heated at 120°C for two hrs., after which they were filtered through #42 Watman paper and diluted to volume with deionized water in 50 ml volumetric flasks.

Wet Ash Digestion (HNO_3 - $HClO_4$)

This technique is a variation of one obtained from J. A. Ryan of the Environmental Protection Agency (personal communication, June, 1975). Following the preliminary treatment of crushing, mixing, screening, and drying, one-gram samples of sludge and two-gram samples of soil were added to 10 ml of concentrated HNO_3 in 50 ml Folin-Wu tubes and allowed to set overnight. The samples were then evaporated to dryness at 120°C on an aluminum block-heater. Additional increments (10 ml) of HNO_3 were added and evaporated until the brown fumes caused by organic matter ceased and white fumes began to evolve. A mixture of 2 ml of 70% $HClO_4$ and 10 ml of concentrated HNO_3 is then added and twice brought to dryness. Fifteen ml of 6 N HCl were added and the tubes were heated for two hours at 120°C. The solution was filtered through #42 Watman paper and brought to volume in a 50 ml volumetric flask with deionized H_2O. Total time of digestion procedure was about 24 hours.

Nitric Acid Extraction

This method was developed by Dr. L. Sommers of Purdue University (personal communication, July 1975). It involves mixing five grams of soil with 50 ml of 6 N HNO_3 and shaking the mixture for 16 hours. The solution was then filtered and diluted to 100 ml.

Wet Ash Digestion (HNO_3 - HCl)

Another wet ash digestion method developed by Van Loon and Lichwa (4) was investigated. It involves adding the sample to an acid mixture of 18 ml of concentrated HCl and 6 ml of concentrated HNO_3. The samples were heated for 30 minutes at 110°C in 50 ml Folin-Wu test tubes on an aluminum block heater. Then they were diluted with 10 ml of water, filtered through Watman #42 paper, and brought to volume in a 50 ml volumetric flask.

Complete Digestion with HF

This method is a variation of one suggested in the Perkin Elmer atomic absorption operations manual. The samples were fired in porcelain crucibles for two-and-one-half hours at 550°C. The material was then placed in teflon evaporating dishes, covered with 10 ml of concentrated HCl and 10 ml of concentrated HF, and evaporated at 80° - 90°C to dryness (about 3 hours). This procedure was repeated two more times. The residue was dissolved with HCl, then poured into a 50 ml volumetric flask and diluted to volume.

Atomic Absorption Analysis

Analyses for cadmium, lead, nickel, copper, and zinc were performed on a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer with a standard air-acetylene burner and hollow-cathode lamps. The settings for the instrument and the other experimental conditions were in accordance with the manufacturer's specifications.

RESULTS AND DISCUSSION

Table I shows the trace element values obtained using three sample preparation techniques along with the analytical statistics. For the most part, dry ashing and wet ashing (HNO_3- $HClO_4$) give comparable values. Application of the statistical "t" test shows that, with two exceptions, the differences between the means of trace element values for samples digested by these two procedures are not significant ($p < 0.05$). The exceptions, lower Ni and Cu values in Englewood sludge digested by wet ashing, may have resulted from spattering during boiling. The reproducibility of dry ashing is better with relative standard deviations below 5% compared to values of up to 11% for wet ashing. It is noted that there was no apparent loss of metals with dry ashing, in spite of the use of high temperatures.

Nitric acid extraction gives lower values than dry ashing. For the most part, the means of trace element values obtained by the two methods are significantly different (Table I).

The HNO_3 - HCl digestion of Dayton sludge results in lower values than dry ashing. Trace element means for three of the five elements are significantly (statistically) different. Complete digestion with HF of Englewood sludge gives values for four of the elements which are not significantly (statistically) different from those obtained by dry ashing. The difference of the means for Ni is just barely significant at the 0.05 level. Considering the values obtained using HF, it appears that both dry ashing and wet ashing (HNO_3- $HClO_4$) extract all of the heavy metals from a sample.

Samples of the National Bureau of Standards Orchard Leaves standard (#1571) were digested by dry ashing and analyzed (Table II). There is very good agreement with the stated N.B.S. values.

CONCLUSIONS

It is concluded that the dry ash digestion method is the best for preparing soil and sewage sludge samples for atomic absorption analysis. The method is faster and easier to perform than the others; analytical precision is good; comparing it with the complete digestion with HF method, it appears to give a good estimate of the total heavy metal contents; and the metal concentrations determined in the N.B.S. standard are in good agreement with N.B.S. published data.

TABLE I. "TOTAL EXTRACTION" OF HEAVY METALS FROM SLUDGES AND SOILS USING THREE DIGESTION PROCEDURES (1)

	DRY ASHING (ppm)			WET ASHING (ppm) (HNO_3 - $HClO_4$)			SIGNIFICANT DIFFERENCE BETWEEN WET ASH. & DRY ASH. (5)	HNO_3 EXTRACTION (ppm)			SIGNIFICANT DIFFERENCE BETWEEN HNO_3 EXTRACT. & DRY ASH. (5)
	$\bar{X}$ (2)	S.D. (3)	R.S.D. % (4)	$\bar{X}$	S.D.	R.S.D. %		$\bar{X}$	S.D.	R.S.D. %	
CADMIUM											
DAYTON SLUDGE	414.0	± 11.0	3.0	422.0	± 7.4	1.7	No	341	± 8.0	2.0	Yes
ENGLEWOOD SLUDGE	6.2	± .06	1.0	5.9	± 0.5	9.3	No	5.4	± 0.2	4.0	Yes
MIAMI SILT LOAM	0.7	± 0.0	0.0	0.7	± 0.07	11.0	No	0.9	± 0.0	0.0	Yes
LEAD											
DAYTON SLUDGE	3080	± 124.0	4.0	3193.0	± 265.0	8.3	No	2852	± 64.0	2.0	No
ENGLEWOOD SLUDGE	221	± 1.6	0.7	223.0	± 6.0	2.7	No	136	± 15.4	11.0	Yes
MIAMI SILT LOAM	24	± 1.2	5.0	24.5	± 1.1	4.3	No	24	± 1.5	6.0	No
NICKEL											
DAYTON SLUDGE	575	± 21.0	3.7	608	± 47.0	7.8	No	527	± 25.0	4.7	No
ENGLEWOOD SLUDGE	1825	± 45.0	2.5	1400	± 21.4	1.5	Yes	1167	± 82.0	7.0	Yes
MIAMI SILT LOAM	18	± 0.0	0.0	16.3	± 0.8	5.0	No	6.7	± 1.2	18.0	Yes
COPPER											
DAYTON SLUDGE	2409	± 19.0	0.7	2309	± 96.0	4.1	No	1972	± 54.0	2.7	Yes
ENGLEWOOD SLUDGE	863	± 45.0	5.0	729	± 13.0	1.8	Yes	557	± 0.0	0.0	Yes
MIAMI SILT LOAM	19.3	± 0.1	0.4	13.9	± 0.9	6.1	No	9.3	± 2.4	26.0	Yes
ZINC											
DAYTON SLUDGE	10030	± 163.0	1.6	10118	± 411.0	4.0	No	4024	± 99.0	2.4	Yes
ENGLEWOOD SLUDGE	965	± 17.0	1.8	910	± 54.0	6.0	No	740	± 34.0	4.6	Yes
MIAMI SILT LOAM	73	± 2.0	2.7	64	± 4.2	7.0	No	26	± 1.7	6.6	Yes

(1) $n = 3$ (no. of replicates)
(2) $\bar{X}$ = MEAN
(3) S.D. = STANDARD DEVIATION
(4) R.S.D.% = RELATIVE STANDARD DEVIATION IN PERCENT
(5) "t" test for significant difference ($p < 0.05$) between means

TABLE II. 'TOTAL EXTRACTION' OF HEAVY METALS FROM NATIONAL BUREAU OF STANDARD'S SAMPLE #1571 USING DRY ASH DIGESTION

ORCHARD LEAVES
(#1571)

	NBS' CONC. (ppm)	OUR MEAN (ppm)
CADMIUM	0.11 ± .02	0.11 ± .01
LEAD	45 ± 3	48 ± 5
NICKEL	1.3 ± 0.2	1.6 ± 0.4
COPPER	12 ± 2.0	12 ± 1.0
ZINC	25 ± ?	25 ± 1.0

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CREDIT

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Cadmium Availability in Soil Treated with a Sludge Enriched with Cadmium During Anaerobic Digestion[1]

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ABSTRACT

Sewage sludge enriched with $CdCl_2$ to a concentration of 1,414 ppm during anaerobic digestion was added to Fox sandy loam in the amount of 8.85 dry tons/acre. The concentration of DTPA-extractable Cd in soil treated with sludge was 17 times greater than that in control soil. Cadmium availability in sludge-amended soil increased following harvest of a 50-day crop of sugar beet (Beta vulgaris L.). The amount of extractable Cd in treated soil was 14% of the total Cd from applied sludge. During anaerobic digestion, Cd (added to sludge as a salt) undergoes chemical changes, being modified into molecular forms less available for chemical extraction. Since there is a positive correlation between Cd uptake by plants and DTPA-extractable Cd from soils, it is believed that Cd uptake values for plants grown on soils to which sludge enriched with Cd after digestion has been added, are unrealistically high.

Additional Index Words: DTPA-extractable Cd, sewage sludge, heavy metals.

INTRODUCTION

Solid and liquid waste disposal is a current problem of major concern in our society. Application of sewage sludge to agricultural soils is an attractive disposal alternative which provides for possible utilization of sludge-borne nutrients and organic matter by recycling them. Utilization of sewage sludge as an agricultural soil amendment has caused concern over possible entry of potentially toxic heavy metals present in sewage sludge into the human food chain and reduced crop yields due to their phytotoxicity (Chaney, 1973). Cadmium appears to be the metal of most concern to human health when applying municipal sludge to agricultural soils (Pahren et al., 1977).

The DTPA (diethylenetriaminepentaacetic acid)-soil test developed by Lindsay and Norvell (1969) is widely used as an index of Zn in the soil available for plant uptake. Due to the chemical similarity of Cd and Zn, Bingham et al. (1976) used DTPA-extractable soil Cd as an index of Cd in soil available for plant uptake. Significant positive correlations between DTPA-extractable soil Cd and Cd content of plants have been reported (Street et al., 1977; MacLean, 1976).

In order to determine whether the cadmium in sewage sludge-amended soils increases Cd content of plants, several investigators have added Cd salts to anaerobically digested sludge before adding the sludges to soils (Bingham et al., 1975; Kirkham, 1975). Anaerobic digestion of sewage sludge is a complex biochemical process in which several groups of facultative and anaerobic organisms simultaneously assimilate and metabolize organic constituents in sludge. During digestion, metals present in sludge may be incorporated into organic compounds. Chemical

availability of metals in soils and their accumulation by plants are dependent upon the molecular species present, not simply the total amount of metal. To test the validity of using freshly added salts in studies of Cd uptake from sewage sludge, an investigation was conducted to evaluate the chemical availability of Cd in soil amended with a sewage sludge enriched with Cd during anaerobic digestion.

MATERIALS AND METHODS

Production of Cd-enriched, anaerobically digested sewage sludge was accomplished using a laboratory designed anaerobic digester (Fig. 1). The digestion chamber was a 235-l plastic garbage can with a plexiglass top, using window putty to provide an airtight seal. Into the digestion chamber was added 32.2 l of anaerobically digested sludge obtained from the anaerobic digestion tanks at the Dayton Municipal Wastewater Treatment Plant (DMWTP), Dayton, Ohio. To this was added 13.7 l of primary, undigested sludge collected from the bottom of the settling beds at the DMWTP, and 2,218 mg $CdCl_2$, in equal increments over a 23-day period. Addition of $CdCl_2$ increased Cd concentration of the sludge by 1,000 ppm, resulting in a final concentration of 1,414 ppm Cd on a dry weight basis. The sludge was stirred constantly at approximately 20 rpm. The digestion chamber, which was partially immersed in a 30-gal metal tub filled with water, was kept at 33°C using a mercurial thermoregulator water heater (Sargent Welch). Digestion was maintained for 26 days. Completion of anaerobic digestion was ascertained when pH increased to 7.4 and percent volatile residues (U. S. Environmental Protection Agency, 1974) decreased from an initial 63% to 47%.

The soil used in the experiment was Fox sandy loam. Soil treated with sludge was amended with liquid sludge equivalent to 8.85 dry tons/acre, and the soil was allowed to dry at room temperature. All soils were fertilized with N-P-K (65, 261 and 261 lbs/acre, respectively), as recommended by the Ohio State University Soil Testing Laboratory, homogenized, and added to 4½" x 4½" plastic pots. Control soil had no

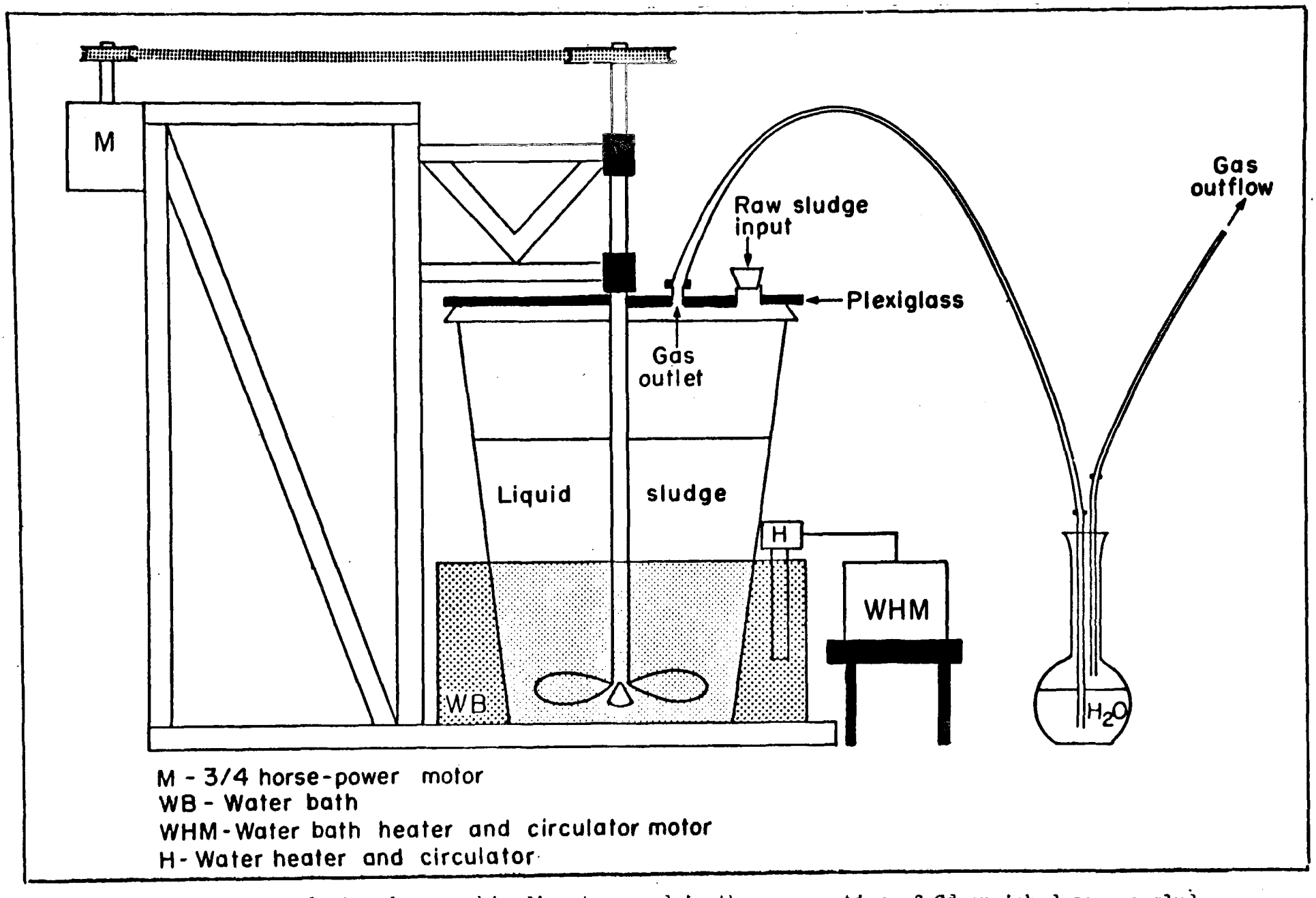

Figure 1. Laboratory designed anaerobic digester used in the preparation of Cd-enriched sewage sludge.

sludge added.

Sugar beet (Beta vulgaris L.) seeds were germinated in vermiculite, transplanted into pots and thinned to two seedlings per pot. Plants were grown in an environmental chamber in a randomized block design in three groups with three fold replication of each soil treatment (18 pots). Plants were watered every other day with deionized water, and once a week given half-strength Hoaglands' solution minus $MnCl_2$, $ZnCl_2$ and $CuCl_2$.

Soil samples were taken from pots prior to planting and following crop harvest. Soils from the three pots in a group of similarly treated soils were combined and thoroughly mixed. Ten-g soil samples from batched soils and 20-ml DTPA-extracting solution, pH adjusted to 7.3 with HCl (Lindsay and Norvell, 1969), were put into 50-ml centrifuge tubes. Tubes were placed horizontally in a reciprocal shaker and shaken for an hour at 280 oscillations per minute. Samples were centrifuged at 3000 X gr. at 24°C for 20 min., filtered through Whatman No. 42 paper, and the filtrate analyzed for Cd by flame atomic absorption spectrophotometry. Determinations of DTPA-extractable Cd from soil samples were performed in triplicate. Soil, sludge and sludge-amended soil parameters are listed in Table 1.

Table 1. Physical and chemical characteristics of Fox sandy loam, Cd-enriched sludge and sludge-amended Fox sandy loam.

Characteristic	Fox sandy loam	Cd-enriched sludge	Sludge-amended Fox sandy loam
Sand*	63%		
Silt*	14%		
Clay*	21%		
Organic carbon†	2%	20.95%	
Cation exchange capacity‡	13.4 meq/100g	28.43 meq/100g	
pH§	6.3	7.4	
Total N¶	0.13%	1.41%	
" P‖	1245 ppm	15540 ppm	
" K**		1278 ppm	
" Ca**		95025 "	
" Mg**		6506 "	
" Na**		425 "	
" Cd**	0.2 "	1414†† "	29.6 ppm
" Cu**	16 "	2298 "	64 "
" Ni**	13 "	472 "	22 "
" Pb**	16 "	3267 "	
" Zn**	45 "	8359 "	219 "

* Day, 1965.
† Allison, 1965.
‡ Chapman, 1965.
§ Peech, 1965.
¶ Bremner, 1965.
‖ Sommers and Nelson, 1975.
** Standard AAS methods.
†† Cd concentration of sludge before adding $CdCl_2$ was 414 ppm.

RESULTS AND DISCUSSION

Addition of Cd-enriched sludge to Fox silt loam increased DTPA-extractable Cd in all soils by a factor of approximately 17 (Table 2). The data also shows greater amounts of extractable Cd after crop harvest than before planting of crops for all soil groups. The chemical availability of Cd in sludge-amended soil increases during time of crop growth. Silviera and Sommers (1977) observed a similar pattern for DTPA-extractable Cd in sludge-amended Fox silt loam. They reported that the amount of Cd extracted increased with time (up to four weeks) in Fox silt loam amended with sludge from Logansport or Kokomo, Indiana. These results may indicate that Cd becomes increasingly available for plant uptake during crop growth on sludge-amended soil and thereby presenting potential hazards to the human food chain.

Determinations have been made of the amount of DTPA-extractable Cd in soils amended with sludge enriched with Cd salts after anaerobic digestion. Street et al. (1977) reported that the DTPA extractant removed 50% of the applied Cd, whereas Bingham et al. (1975) reported the removal of 60% of the applied Cd for soils treated with sludge enriched with $CdSO_4$ after anaerobic digestion. In the present study, the amount of extractable Cd (2.17μgCd/mlDTPA) was 14% of the total Cd (29.6μgCd/g soil) in the sludge-amended soil (Table 2). When Cd is added to sludge during digestion, the subsequent availability of this Cd after land application of the sludge is much less than Cd availability from sludge to which Cd was added after digestion.

The relative availability of metals in sludge-amended soil is dependent upon the physical and chemical properties, and biological

Table 2. DTPA-extractable Cd (in ppm) from Fox sandy loam and sludge-amended Fox sandy loam before and after cropping soil with sugar beet (n = 3). *

Group	Fox sandy loam		Sludge-amended Fox sandy loam	
	Before crop	After crop	Before crop	After crop
1	0.15[a]	0.13[a]	2.29[b]	2.60[c]
2	0.12[a]	0.13[a]	1.88[b]	2.35[c]
3	0.19[a]	0.11[a]	2.34[b]	2.72[c]

* Mean values, within a group (rows), followed by the same letter (a-c), did not significantly differ at the 5% level according to Duncan's Multiple Range Test.

characteristics of the soil. An important factor in determining the availability of metals in soils is the particular molecular species in which the metal exists. When Cd salt is added to sludge after anaerobic digestion, much of the Cd may exist in ionic complexes or form chelates which are easily reversible. This would explain the high percentage of extractable Cd in soils amended with sludge enriched with Cd salt after anaerobic digestion. When Cd salt is added to sludge during anaerobic digestion, the assimilation of Cd by microorganisms and its subsequent involvement in metabolic reactions of these organisms may modify the molecular species of Cd present in the sludge. If during anaerobic digestion most of the Cd is chelated by compounds with many functional groups or is covalently bonded in nitrogenous compounds, this would explain why Cd in soils amended with sludge enriched with Cd during digestion is relatively unavailable.

If metal enrichment of sludge during digestion simulates natural conditions with respect to metal extractability whereas metal added to sludge after digestion is readily available from sludge-soil mixtures, then the validity of results obtained from experiments performed using the latter experimental design should be carefully scrutinized as to their relevance. Data presented here seems to suggest that adding metal salts to digested sludge gives little indication of the availability to plants of those metals when incorporated during the digestion process.

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Heavy Metal Uptake by Sugar Beet and Spinach From Three Sludge-Amended Soils[1]

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ABSTRACT

This study compares uptake of zinc, copper, nickel, cadmium and lead by sugar beet and spinach from Miami silty clay loam, Fox sandy clay loam, and Ross sandy clay loam, after amendment with sewage sludge. Maximum and half-maximum allowed amounts of municipal (Dayton, Ohio) and suburban (Englewood, Ohio) sludge were applied. Dayton sludge has a heavy metal content greater than 400 ppm for all the elements, with a maximum of 9300 ppm for zinc and a Cd/Zn ratio of 4.3%. Englewood sludge ranges from 6 ppm for cadmium to 1860 ppm for nickel. Any additional of Dayton sludge reduced sugar beet and spinach yields, whereas added Englewood sludge increased shoot dry weight of both species.

The pattern of heavy metal uptake, which differed for each metal, was complicated by interactions among soil type, plant species and plant part. Addition of maximum and half-maximum amounts of Dayton sludge caused uptake of zinc in roots and shoots of both plant species in all soils. No other metal uptake was this extensive. Englewood sludge did not increase any metal uptake in both plant species and in all the soils. In fact, neither plant species accumulated more cadmium or lead than controls in any soil. A model of cadmium uptake is presented. Available cadmium, as extracted with DTPA, has the strongest negative correlation with plant dry weight for both species. The strongest negative correlations between dry weight and plant heavy metal content is with cadmium for spinach and with copper for sugar beets. After cadmium, lead in the soil explains the greatest amount of variation in dry weight. Soil cadmium also strongly correlates with most heavy metal contents in plants. Copper strongly correlates with some plant metal contents, particularly in the roots.

The complex interactions suggest that caution is needed when decisions are made for land application of sewage sludge.

Introduction

As more and better treatment of sewage is introduced into municipalities, and environmental awareness increases, disposal of sludge becomes a greater problem. Landfill and lagooning are becoming too costly as land prices rise. Municipal concern coupled with increased fertilizer costs have made land application of sludge an apparently attractive alternative. Apart from the costs of application, the main drawback to increased use of municipal sludge as a soil conditioner is the potential hazard arising from the heavy metal content of most urban sludges which originate partly from industrial processes. Besides these hazards, salts, pathogens, and carcinogenic chemicals (e.g. pesticides, p.c.b.'s) are also present (Furr et al., 1976). We shall address only the problem of the heavy metals: zinc, copper, nickel, cadmium, and lead.

A primary concern in land application is the risk of introducing potentially dangerous chemicals into the food chain, eventually adding to the heavy metal intake of the human population. Prior to this result, farmers are concerned about any serious reduction in crop yields from phytotoxic heavy metals, especially zinc, copper, and nickel. The Ohio Agricultural Research and Development Center (O.A.R.D.C.) has proposed a formula for land application (Grooms, 1975) which takes into account not only the heavy metal content of the sludge but the pH and cation exchange capacity (C.E.C.) of the soil. Because the problem is exceedingly complex, general guidelines must be applied with caution since heavy metal uptake can be affected by soil moisture, pH, C.E.C., available soil phosphate, organic matter, and the presence of other metals (Dowdy and Larson, 1975). Since different tolerances to heavy metals have been demonstrated (Gaynor & Halstead, 1976) as well as differential accumulation in tissues of the same crop (Williams and David, 1977), the crop being grown is

also an important factor.

This study attempts to compare the heavy metal uptake of spinach and sugar beet from three important agricultural soils amended with a municipal (Dayton, Ohio) and suburban (Englewood, Ohio) sewage sludge.

TABLE 1. PHYSICAL AND CHEMICAL PROPERTIES OF TEST SOILS AND SLUDGES

	SOIL			SLUDGE	
	FOX	MIAMIAN	ROSS	DAYTON	ENGLEWOOD
TEXTURE	SANDY CLAY LOAM	SILTY CLAY LOAM	SANDY CLAY LOAM	--	--
% Sand	67.5	34.3	60.1	--	--
% Silt	12.6	40.0	17.1	--	--
% Clay	19.9	25.7	22.9	--	--
Exchangeable K * (ppm)	60.5	200	187	111	625
Exchangeable Ca * (ppm)	1,415	1,440	2,290	3,955	3,360
Exchangeable Mg * (ppm)	378	307	246	444	1,001
C.E.C. * (m.e./100g)	15.9	22.9	15.5	24	29
Org. C. (%)	2.0	2.9	1.8	20.9	22.4
N %	0.12	0.21	0.12	1.4	2.5
Available P * (ppm)	15.5	22	59	78	78
pH *	7.1	6.4	7.5	6.6	6.8

* Data from Ohio State University Soil Test Lab

Methods and Materials

Three agricultural soils were used in the experiment: Fox, a sandy clay loam; Miami, a silty clay loam; and Ross, a sandy clay loam. Surface (0-12 inch) samples were collected from sites selected as typical in the Montgomery County soil survey (U.S.D.A. Soil Conservation Service, 1976). Air-dried sludge was collected from drying beds at the Dayton Municipal and Englewood treatment plants. Dayton sludge is a mixture of domestic and industrial effluent, while the Englewood sludge is primarily domestic. A nickel plating plant is the only significant industrial source in the Englewood system. Table 1 gives properties of soils and sludges.

Air-dried sludge and soil samples were screened through a 2mm sieve and measured amounts of dried sludge added to the soil to approximate the recommended applications (Table 1a) as calculated using the formula proposed by the O.A.R.D.C.

Table 1a. Maximum Sludge Application to Test Soils

Soil Type	Dayton Sludge T/acre	Dayton Sludge g/pot	Englewood Sludge T/acre	Englewood Sludge g/pot
Fox	17.7	38.4	20.1	43.8
Miami	25.1	54.6	28.5	61.9
Ross	34.5	75.0	39.2	85.3

Five treatments were performed with each soil type: Control (no sludge), Dayton maximum, Dayton $\frac{1}{2}$ maximum, Englewood maximum, Englewood $\frac{1}{2}$ maximum. There were three replicates of each treatment.

Seeds of sugar beet (Beta vulgaris, L. cv. USH 20) and spinach (Spinacia oleracea) were started in moist vermiculite. After emergence, seedlings were transferred to pots and later thinned to two plants per pot. Pots were placed

randomly in three growth chambers with a thin layer of perlite added to the surface of each pot to reduce evaporation. Plants were maintained under a regime of 12 hr. light at 25°C and 12 hr. dark at 17°C. A light intensity of approximately 1800-2000 f.c. was maintained at the top of the plants by lowering the pots as they grew.

Macronutrient levels of test soils were measured at the Ohio State University Soil Testing Laboratory and before the first planting, N-P-K solution was added to the level recommended. Plants were watered with de-ionized water every other day and weekly with 50 ml of $\frac{1}{2}$ strength Hoagland's nutrient solution made up without Mn, Zn, and Cu. To ensure better growth, 63% more nitrogen as NH_4NO_3 was added to the nutrient solution.

After seven weeks growth, plant samples were harvested, soil removed carefully from roots, and separated into shoot and root portions. Because some samples were small and both plants did not always grow equally, biomass determinations were determined on a per pot basis by combining samples of both plants in the pot. Plant material was dried overnight at 85°C and weighed, then ground in a Wiley mill and stored in vials.

Sample aliquots were dry-ashed for eight hours at 550°C, digested for two hours in 3 N HCl, and brought to volume with de-ionized water. The digested sample was analyzed on a Perkin-Elmer 603 Atomic Absorption Spectrophotometer. Low concentrations of heavy metals were assayed using the graphite furnace flameless AAS (Perkin-Elmer Model HGA 2100).

Soil and sludge pH was measured on a pH meter using a 1:2 soil:0.01 M $CaCl_2$ slurry. Organic carbon was determined by the Walkley-Black method. Total phosphorus was determined according to Sommers and Nelson (1972). Total nitrogen was measured by the Dumas method on a Coleman Model 24 Nitrogen Analyzer. Total heavy metal content was measured by the dry ash method described by Ritter et al. (1978). Available heavy metals were determined by extraction with DTPA (Brown, 1978).

Results and Discussion

Table 2 shows the heavy metal content of the sludges and soils used in the experiment. The soils show very similar levels while the sludges are quite different. The Dayton sludge has much higher total heavy metal concentration for all metals except Nickel, which is greater in the Englewood sludge. Chaney (1973) considers a Cd/Zn ratio of total metal content in sludge of less than 0.5% as being the safe limit for application to agricultural land. Dayton sludge has a Cd/Zn ratio well above Chaney's limit while Englewood sludge is just at this limit. When total heavy metal content of sludge is considered, the order of concentration in Dayton sludge is: zinc > lead > copper > nickel > cadmium. For Englewood the order is: nickel > zinc > copper > lead > cadmium. In both sludges, the concentration of cadmium is smallest.

Experimental soils used in the potting experiments were extracted with DTPA to determine heavy metal availability. The results of this extraction are given in table 3. Treatment levels are those given in table 1a. Data for DTPA-extractable metals show that with Dayton sludge in all soils zinc is most available, then copper; whereas in the total content, lead followed zinc in order of concentration. In the DTPA extracts lead could be third highest, as in Fox soil, or lowest, as it was with Miami and Ross soils. Cadmium was least concentrated in extracts of Fox soil and in the lower application rates on the other soils. Englewood sludge extracts more closely follow the concentration order of the total sludge values except that copper and zinc are either reversed or equal in their relative concentrations. Copper seems more available than zinc in Englewood sludge, relative to its concentration in the sludge. Lead and cadmium maintain the same order of concentration in both total and extract values. Tables 4, 5, 6 give the dry weight values and heavy metal

TABLE 2. TOTAL HEAVY METAL CONTENT (ppm) OF SLUDGES AND MONTGOMERY COUNTY SOILS

SOILS	ZINC	COPPER	NICKEL	CADMIUM	LEAD	Cd/Zn (%)
Miami	68	17	18	0.3	22	
Fox	45	16	13	0.1	16	
Ross	60	14	14	0.2	18	
SLUDGES						
Dayton	9300	2300	450	400.0	3300	4.3
Englewood	1120	870	1860	6.0	240	0.5

TABLE 3. DTPA-EXTRACTABLE SOIL HEAVY METAL CONTENT (ppm) OF CONTROL AND SLUDGE-AMENDED SOILS

	ZINC	COPPER	NICKEL	CADMIUM	LEAD
Fox Soil					
Control	1.4	0.60	0.18	0.06	0.65
Dayton	41.7	7.39	5.04	3.63	6.46
Dyt/2	22.9	5.01	1.86	1.66	4.21
Englewood	4.0	5.05	10.90	0.12	0.79
Eng/2	2.1	1.64	1.51	0.08	0.73
Miami Soil					
Control	2.3	0.73	0.28	0.09	1.48
Dayton	50.7	7.84	8.27	4.65	1.90
Dyt/2	34.3	6.68	3.83	3.14	3.33
Englewood	6.0	6.71	17.00	0.14	1.59
Eng/2	4.3	4.44	8.60	0.13	1.61
Ross Soil					
Control	2.5	1.12	0.19	0.07	1.23
Dayton	87.9	8.91	6.50	5.88	4.37
Dyt/2	31.8	6.59	3.02	2.69	6.26
Englewood	8.2	9.29	36.90	0.20	1.43
Eng/2	4.6	4.76	9.40	0.09	1.44

TABLE 4. PLANT YIELD (dry wt./pot of 2 plants) AND HEAVY METAL UPTAKE (μg/gm dry wt.).

DATA ARE MEANS OF 3 REPLICATES ON ROSS SOIL AMENDED WITH SLUDGE.

	DRY WT.	ZINC	COPPER	NICKEL	CADMIUM	LEAD
SPINACH						
Shoot						
Dayton	*0.073 c	237 a	50 a	62 a	4.4 a	12 a
Englewood	0.811 bc	78 b	15 a	23 a	0.6 b	15 a
Dyt/2	0.138 c	138 ab	19 a	35 a	4.2 a	17 a
Eng/2	1.634 b	63 b	15 a	18 a	0.3 b	11 a
Control	3.393 a	75 b	14 a	18 a	0.3 b	12 a
Root						
Dayton	0.019 b	280 a	144 a	307 a	8.8 a	47 a
Englewood	0.173 ab	152 ab	52 b	144 ab	1.1 b	22 b
Dyt/2	0.036 b	184 ab	72 b	46 b	6.8 a	27 b
Eng/2	0.474 ab	75 b	26 b	97 ab	0.6 b	17 b
Control	0.619 a	70 b	28 b	21 b	0.5 b	22 b
SUGARBEET						
Shoot						
Dayton	0.059 c	285 a	196 a	22 a	2.5 a	41 a
Englewood	3.673 b	33 c	16 b	18 ab	0.1 b	9 a
Dyt/2	0.205 c	233 b	143 ab	19 ab	2.0 a	39 a
Eng/2	4.712 a	32 c	14 b	13 ab	0.1 b	10 a
Control	4.253 ab	32 c	28 b	8 b	0.5 b	12 a
Root						
Dayton	0.039 c	222 a	286 a	75 a	3.2 a	18 a
Englewood	0.695 bc	41 b	33 b	33 b	0.2 a	21 a
Dyt/2	0.095 c	151 a	74 b	22 b	1.7 a	49 a
Eng/2	1.850 ab	37 b	23 b	25 b	0.2 a	14 a
Control	2.728 a	24 b	17 b	11 b	.6 a	7 a

* Means in each column (of 5) with same letter are not significantly different at 5% level by Duncan's Multiple Range Test.

TABLE 5. PLANT YIELD (dry wt./pot of 2 plants) AND HEAVY METAL UPTAKE (μg/gm dry wt.). DATA ARE MEANS OF 3 REPLICATES ON MIAMI SOIL AMENDED WITH SLUDGE.

	DRY WT.	ZINC	COPPER	NICKEL	CADMIUM	LEAD
SPINACH						
Shoot						
Dayton	0.068 *c	233 a	38 a	24 b	6.0 ab	128 a
Englewood	5.734 a	189 a	18 b	40 a	0.5 b	9 a
Dyt/2	0.562 c	175 a	17 b	10 c	13.1 a	7 a
Eng/2	5.851 a	128 a	12 b	23 b	0.5 b	9 a
Control	3.224 b	148 a	11 b	5 c	0.4 b	7 a
Root						
Dayton	0.016 b	256 ab	98 a	51 c	13.7 a	114 a
Englewood	0.973 ab	182 bc	92 a	332 a	2.0 b	22 b
Dyt/2	0.150 ab	271 a	80 a	40 c	4.1 b	24 b
Eng/2	0.906 ab	105 cd	37 b	150 b	0.6 b	13 b
Control	1.221 a	92 d	26 b	17 c	0.6 b	15 b
SUGARBEET						
Shoot						
Dayton	0.552 d	432 a	138 a	17 a	2.4 b	18 a
Englewood	8.425 d	77 c	13 b	22 a	0.2 b	8 b
Dyt/2	1.492 a	265 b	28 b	31 a	6.0 a	10 b
Eng/2	7.317 b	82 c	18 b	18 a	0.1 b	7 b
Control	6.267 c	96 c	21 b	9 a	1.6 b	10 b
Root						
Dayton	0.127 b	258 a	100 a	43 a	9.2 a	52 a
Englewood	3.182 a	59 b	33 c	40 a	0.4 b	13 b
Dyt/2	0.715 b	219 a	65 b	32 a	8.0 a	18 b
Eng/2	3.613 a	49 b	24 c	27 a	0.2 b	15 b
Control	3.828 a	34 b	20 c	27 a	1.0 b	9 b

* Means in each column (of 5) with same letter are not significantly different at 5% level by Duncan's Multiple Range Test.

TABLE 6. PLANT YIELD (dry wt./pot of 2 plants) AND HEAVY METAL UPTAKE (μg/gm dry wt.

DATA ARE MEANS OF 3 REPLICATES ON FOX SOIL AMENDED WITH SLUDGE.

	DRY WT.	ZINC	COPPER	NICKEL	CADMIUM	LEAD
SPINACH						
Shoot						
Dayton	0.081 d*	136 ab	48 a	28 ab	2.8 a	33 a
Englewood	5.322 a	116 ab	15 a	39 a	0.3 c	12 b
Dyt/2	0.462 d	172 a	20 a	12 b	0.9 b	10 b
Eng/2	3.639 b	83 b	14 a	18 ab	0.5 c	10 b
Control	2.53 c	58 b	12 a	10 b	0.3 c	12 b
Root						
Dayton	0.016 c	130 ab	54 ab	46 b	10.7 a	62 a
Englewood	1.162 a	131 ab	77 a	314 a	0.6 a	23 a
Dyt/2	0.151 bc	164 a	43 bc	33 b	3.1 a	26 a
Eng/2	0.713 abc	100 bc	49 bc	114 b	0.7 a	20 a
Control	0.742 ab	68 c	26 c	19 b	0.5 a	19 a
SUGARBEET						
Shoot						
Dayton	0.195 c	274 a	110 a	36 a	6.0 a	8 a
Englewood	5.420 ab	48 b	16 b	24 a	0.1 b	7 a
Dyt/2	1.103 c	235 a	41 b	42 a	1.5 b	46 a
Eng/2	6.530 a	51 b	18 b	18 a	0.1 b	9 a
Control	3.973 b	45 b	31 b	28 a	0.9 b	32 a
Root						
Dayton	0.094 b	285 a	539 a	90 a	18.5 a	8 b
Englewood	2.659 a	57 b	36 b	41 b	0.2 b	14 b
Dyt/2	0.562 b	241 a	82 b	43 b	19.0 a	62 a
Eng/2	3.790 a	40 b	23 b	29 b	0.1 b	7 b
Control	3.663 a	35 b	22 b	18 b	0.7 b	8 b

* Means in each column (of 5) with the same letter are not significantly different at 5% level by Duncan's Multiple Range Test.

contents of harvested shoots and roots of sugar beets and spinach grown in three soils amended with the two sludges.

Dry Weight Results

An analysis of variance was performed on the data and we shall mention only those effects that are statistically significant at the 5% level of probability by Duncan's Multiple Range Test. For all soils, sugar beet and spinach shoot and root dry weight decreased with the addition of more Dayton sludge in comparison to the weight of control plants without sludge. With Englewood sludge, sugar beet and spinach shoot dry weight increased with greater additions of sludge to Miami and Fox soils. On sludge-amended Ross soils, shoot dry weight of spinach and sugar beets was equal to or less than that of the control plants. Root growth was less consistent. In all soils, root dry weight was equal to or less than controls with all treatments with Englewood sludge.

Heavy Metal Uptake

The plant heavy metal uptake pattern, as seen in tables 4, 5, 6, is quite complicated and there is much variation between soils, sludges, as well as species and plant parts. Considering zinc first, for all soils treated with Dayton sludge both sugar beet and spinach accumulated more zinc than control plants. With Englewood sludge, only in Fox soil was there a greater concentration of zinc in plants given the maximum treatment, compared to controls with no sludge added. For copper, there is greater diversity. Spinach shoots from Ross and Fox soils did not accumulate more copper than controls even with maximum additions of Dayton sludge. All other plants given maximum Dayton sludge treatment accumulated more copper than controls. In plants treated with Englewood sludge, only spinach roots accumulated more copper than control plants.

In contrast to other metals, nickel concentration of Englewood sludge was greater than that of Dayton sludge. With Englewood sludge treatment, in spite of the greater concentration of nickel, only spinach shoots and roots grown on Miami and Fox soil accumulated higher nickel contents than controls. The results of the Dayton sludge treatment are rather different. Maximum Dayton sludge treatment produced a nickel uptake greater than that of control plants in sugar beet roots in Fox soil and in both shoots and roots in Ross soil. With the same treatment, only shoots of spinach plants in Ross and Miami soils accumulated more than controls. When treated with maximum Dayton sludge, all plants accumulated more cadmium than controls with the exception of sugar beet roots on Ross soil and spinach roots on Fox soil. Concerning cadmium and lead, treatment with any amount of Englewood sludge did not produce a plant uptake of either metal greater than that of the controls. Maximum Dayton sludge addition to Miami soil caused both shoots and roots of sugar beets and spinach roots to accumulate more lead than controls. On Ross soil only spinach roots and only spinach shoots on Fox soil accumulated more lead than controls with maximum Dayton treatment.

Table 7 gives results of an analysis of variance of yield and heavy metal uptake. It underlines the complicated interactions outlined above. Because of the numerous higher order interactions, few conclusions can be drawn about the first order effects. Since there are significant four-way interactions for plant dry weight and plant content of copper and nickel, it is not possible to assume that main effects are valid for these variables. Plant cadmium has significant three-way interactions and so valid inferences cannot be made about its main effects. That leaves plant zinc and lead and we can conclude from this analysis that soil type has a significant effect on plant zinc. Similarly, for plant lead we can conclude only that plant part, i.e., whether root or

TABLE 7. RESULTS OF ANALYSIS OF VARIANCE OF YIELD AND HEAVY METAL UPTAKE

SOURCE OF VARIATION	PLANT DRY WEIGHT	PLANT CONTENT ZINC	COPPER	NICKEL	CADMIUM	LEAD
MAIN EFFECTS						
V1 Species	***	NS	***	***	NS	NS
V2 Treatment	***	***	***	***	***	***
V3 Soil	***	***	NS	NS	*	NS
V4 Plant Part	***	NS	***	***	***	NS
2-WAY INTERACTIONS						
V1 x V2	***	***	***	***	NS	***
V1 x V3	*	NS	NS	NS	***	NS
V1 x V4	NS	**	NS	***	NS	NS
V2 x V3	***	NS	NS	***	*	***
V2 x V4	***	NS	**	***	***	NS
V3 x V4	***	NS	NS	NS	*	NS
3-WAY INTERACTIONS						
V1 x V2 x V3	*	NS	NS	**	**	NS
V1 x V2 x V4	NS	NS	NS	***	*	NS
V1 x V3 x V4	NS	NS	*	NS	NS	NS
V2 x V3 x V4	***	NS	*	**	**	NS
4-WAY INTERACTIONS						
V1 x V2 x V3 x V4	**	NS	**	*	NS	NS

$p = < .001$ ***

$p = < .01$ **

$p = < .05$ *

NS = Not significantly different

shoot, has no significant effect on plant lead content. It is clear that, because of the complicated interactions, analysis of variance is not too useful. Regression analysis will be used to further elucidate the relationship between soil heavy metal content and plant uptake of heavy metals.

In an attempt to further explain the effect of heavy metal uptake and soil availability of heavy metals on plant dry weight, simple correlation coefficients of plant dry weight as dependent variables versus plant and soil available heavy metal contents as independent variables are shown in Table 8. The available, i.e., DTPA-extractable cadmium in the soil has the strongest negative correlation with plant dry weight followed by zinc then lead, for both species tested. Correlations between plant heavy metal content and dry weight are different. Spinach dry weight of both shoots and roots has the strongest negative correlation with plant cadmium followed by copper, then lead and zinc, while for sugar beets it is copper followed by zinc then cadmium and lead.

Model of Cadmium Uptake

If we consider Fox soil, with the lowest sludge application rate allowed (see Table 1a), and cadmium, which seems to have the strongest correlation with plant dry weight and is the greatest danger to the human food chain, a model for the uptake of cadmium can be constructed (Table 9). Notice that about 60% of the soil cadmium in the control soil is available (as determined by DTPA-extraction), and about 20% of the cadmium in the maximum Dayton treatment and about 32% of that from maximum Englewood treatment is available. The concentration of cadmium in the spinach shoots is always less than that of the roots. In shoots of sugar beet, the control has a slightly higher concentration than the root, but in both sludge-treated soils the root concentration is higher than the shoots. The transport index, i.e., the amount of cadmium (total μg) in the shoot compared to the total present in the plant, for spinach is .58 with

TABLE 8. SIGNIFICANT SIMPLE CORRELATION COEFFICIENTS (r - 1% level) OF DRY WEIGHT VERSUS PLANT HEAVY METAL CONTENT AND DTPA-EXTRACTABLE HEAVY METAL CONTENT OF ALL SOILS (LOG TRANSFORMATION OF ORIGINAL DATA)

	ZINC		COPPER		NICKEL		CADMIUM		LEAD	
Dry Wt.	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil
Spinach Shoots	N.S.	-.852	-.535	-.643	N.S.	N.S.	-.720	-.887	-.448*	-.757
Spinach Roots	-.453*	-.795	-.424*	-.639	N.S.	N.S.	-.785	-.835	-.489*	-.723
Sugar-beet Shoots	-.759	-.907	-.913	-.578	N.S.	N.S.	-.688	-.912	-.457*	-.820
Sugar-beet Roots	-.827	-.900	-.878	-.753	-.461*	N.S.	-.628	-.915	-.466*	-.805

* = $p < .05$

TABLE 9. CADMIUM UPTAKE MODEL FOR FOX SOIL WITH MAXIMUM SLUDGE APPLICATIONS.

(Cd values in ppm)

		CONTROL	DAYTON	ENGLEWOOD
SOIL	Total	0.1	17.7	0.38
	DTPA extract	0.06	3.63	0.12
SPINACH	Root	0.5	10.7	0.6
	Shoot	0.3	2.8	0.3
T. I.		.67	.58	.70
SUGARBEET	Root	0.7	18.5	0.2
	Shoot	0.9	6.0	0.1
T. I.		.58	.40	.50

maximum Dayton treatment and .70 for Englewood maximum treatment. For sugar beets, the indices are .40 and .50 respectively. In both species, plants given Englewood treatments had a higher T.I. T.I. values were determined for all treatments but no trends across all soils or species can be seen. Each metal has a different pattern of movement, depending on the soil or sludges involved.

In an attempt to analyze possible antagonistic and synergistic effects between available heavy metals in the soils and their uptake by plants, stepwise multiple regression analysis was done on the experimental data, i.e., available heavy metal content of the soil as independent variables versus plant dry weight and plant heavy metal contents. To increase homogeneity of the variance, log transformation of the data was performed. Each line on Table 10 is a model which shows the most important independent variables that explain the variation in the given dependent variable. This analysis reflects what was stated earlier, that cadmium in the soil has the strongest negative correlation with dry weight. With all plant parts, the metal in the soil that next explains the greatest amount of variation in dry weight, after soil cadmium, is available lead. It is somewhat surprising that, except for plant cadmium, the strongest correlations do not exist between the available heavy metal in the soil and that metal content in the plant. For example, zinc is the metal with the greatest concentration in sludge-amended soils, yet the strongest correlation with zinc in all plant parts but spinach roots is with cadmium in the soil. Copper content of the plant was most strongly correlated with available cadmium except for spinach roots where the strongest correlation was with available copper. More than 70% of the variation in plant copper in sugar beet was explained by soil cadmium. Plant nickel was most strongly correlated with available copper in spinach shoots (12% of the variation explained), nickel in spinach roots (24% of variation), lead in sugar beet shoots (12% of variation) and copper in sugar beet roots

TABLE 10. SIGNIFICANT VARIABLES OF THE MULTIPLE REGRESSIONS OF AVAILABLE HEAVY METALS IN SOIL ON PLANT DRY WEIGHT AND HEAVY METAL CONTENT (LOG TRANSFORMATION OF ORIGINAL DATA)

(Dependent Variable)	AVAILABLE HEAVY METAL IN SOIL (INDEPENDENT VARIABLES) Zn	Cu	Ni	Cd	Pb
Spinach Shoot					
Plant					
Dry weight				*** (79)[1]	**[2]
Zinc				*** (31)	
Copper				*** (40)	
Nickel		* (12)			
Cadmium	**			*** (65)	
Lead	*			* (13)	
Spinach Root					
Plant					
Dry weight				*** (70)	*
Zinc		*** (42)	*		
Copper	*	*** (36)			
Nickel			*** (24)		
Cadmium		*		*** (65)	
Lead				*** (41)	
Sugarbeet Shoot					
Plant					
Dry weight				*** (83)	***
Zinc	***			*** (74)	*
Copper		*		*** (71)	*
Nickel					* (12)
Cadmium	*	*		*** (50)	*
Lead	* (12)				
Sugarbeet Root					
Plant					
Dry weight				*** (84)	***
Zinc	*			*** (73)	**
Copper				*** (72)	***
Nickel		*** (35)			
Cadmium	*			*** (46)	*
Lead		*** (27)			

[1]Coefficient of determination (% of variation explained by linear regression of independent variable with highest F value against dependent variable).

[2]Independent variables that account for a significant amount of variation, using a stepwise regression.

(35% of variation). Plant cadmium was most strongly correlated with available cadmium in all plants and explained 65% of the variation in spinach and nearly 5% in sugar beet. Plant lead was strongly correlated with soil cadmium in spinach and available zinc with sugar beet shoots (12% of variation explained) and with available copper in their roots (which explained 27% of the variation). Besides these simple correlations, other soil metals appeared to account for significant amounts of variation in the dependent variables. For example, available lead accounts for significant amounts of the variation in all plant dry weight in addition to the amount explained by soil cadmium. In this regard, the possible synergistic effect of soil lead and soil cadmium on sugar beet yield and heavy metal uptake may be suggested by the significant amount of variation which is accounted for by soil lead.

The explanation for these correlations is not easy to make but may be indicative of a high degree of interactions among these metals in the soil. The main conclusion that seems possible from the data presented here is that any attempt to apply sewage sludge to land used for cropping must be approached with caution. There is no simple formula that can apply in all cases and each situation must be examined separately with careful consideration given to the heavy metal content of the sludge, the chemical properties of the soil, the type of crop grown and which part of the plant is being utilized for animal or human consumption.

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PLANT UPTAKE AND CHEMICAL EXTRACTABILITY OF HEAVY METALS AND PLANT GROWTH ON SOIL TREATED WITH Cd-ENRICHED SEWAGE SLUDGE AS INFLUENCED BY PHOSPHATE

KEY WORDS: Heavy metals, sewage sludge, plant uptake, phosphate, DTPA-extractable metals

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ABSTRACT

Sugar beets (Beta vulgaris L.) were grown on Fox sandy loam amended with sewage sludge which was enriched with $CdCl_2$ to 1413 ppm during anaerobic digestion. Sludge was applied in amounts equal to 0 and 8.85 dry tons/acre and phosphate in amounts of 0, 261 and 522 lbs/acre. Plants were raised for 50 days in growth chambers, harvested, and divided into roots and shoots. Dry weights and Zn, Cu, Ni, Cd and Pb concentrations in plant tissues were determined. Addition of sludge to soil reduced beet growth and increased root Zn, Cu, Cd and Pb and shoot Cd concentrations. Roots accumulated greater Cu, Ni, and Pb concentrations than shoots of plants grown in sludge-amended soil. Phosphate addition to soil reduced shoot Zn concentrations but did not consistently affect plant uptake of other metals. DTPA-extractable Zn, Cu, Ni and Cd increased with sludge addition to soil while phosphate treatments did not affect the amounts of extractable soil metals. Cropping sludge-amended soil reduced extractable Zn, concomitant with increasing extractable Cu and Cd. This suggests that metals exhibit differential chemical reactions in sludge-amended soil changing their availability. Available soil Cu had the most influence on growth and heavy metal uptake by beet roots. Possible synergisms between extractable Cu and Zn, Cd and Pb uptake by beet roots suggest that addition of sludge low in Zn, Cd and Pb concentrations but high in Cu concentration to soils cropped with root vegetables may increase the amount of Zn, Cd and Pb in the human food chain.

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INTRODUCTION

Disposal of sewage sludge from industrial centers is a growing problem for municipalities. Recent attempts at recycling municipal sewage sludge as an agricultural soil amendment has caused concern over potentially toxic heavy metals present in sludge. These metals can enter the human food chain and can reduce crop yields due to their phytotoxicity[2,3]. The heavy metals of primary concern in sewage sludge are Zn, Cu, Ni, Cd and Pb. Wide variations in concentrations of heavy metals in sewage sludge have been reported in a review by Page and Chang[4]. Metal content of sewage sludge appropriate for agricultural land application listed by Chaney[3] are (in μg/g dry weight): Zn <2,000; Cu <800; Ni <100; Cd <0.5% of Zn; Pb <1,000. Few municipal sewage sludges with industrial influents are able to meet these criteria of heavy metal content[5,6].

Diethylenetriaminepentaacetic acid (DTPA)-extractable heavy metal values have been used as an index for heavy metal availability for plant uptake from soils amended with sewage sludge[7]. Addition of sewage sludge to soils increases the total and DTPA-extractable metal content of soils. Significant positive correlations between extractable metals and metal accumulation by plants grown on sludge-amended soils have been observed by Gaynor and Halstead[8]. They also reported that heavy metal uptake by lettuce and amounts of metals extracted from soils were greater for coarse-textured sandy loam than for fine-textured clay to which sludge was added.

Conflicting observations of the effects of phosphate on heavy metal uptake by plants have been reported. Chaney[3] states that increasing amounts of phosphate applied to soil decreases stunting of plant growth caused by excessive levels of toxic metals in soils. Both decreasing[2,9] and increasing[10] metal uptake in plants with increasing phosphate additions to soils has been reported.

In view of the above considerations, this investigation with sugar beet was

conducted to: (1) study the effects of phosphate on crop growth; (2) to determine Zn, Cu, Ni, Cd and Pb content in different plant tissues; (3) to measure chemical extractability of Zn, Cu, Ni and Cd from soil amended with sewage sludge enriched with Cd; and (4) to assess effects of crop growth on the chemical extractability of Zn, Cu, Ni and Cd from sludge-amended soil.

MATERIALS AND METHODS

Sewage Sludge

Cadmium-enriched sewage sludge was anaerobically digested in the laboratory. Addition of $CdCl_2$ increased Cd concentration of the sludge by 1,000 ppm on a dry weight basis. Digestion procedures and determination of digestion completion were according to Brown et al.[11]

Soil and Sludge Analysis

Soil and sludge parameters were determined as in Brown.[12] Total and extractable metal determinations were also according to Brown.[12] Results are listed in Table 1.

Plant Growth

Sludge was added to Fox sandy loam, a Typic Hapludalf, in amounts equaling 0 and 8.85 dry tons/acre. Soils were fertilized with 65 lbs/acre N and 261 lbs/acre K as recommended by the Ohio State University Soil Testing Laboratory. Phosphate treatments were 0, 261 and 522 lbs/acre. The recommended amount of phosphate fertilizer was 261 lbs/acre.

Sugar beet (Beta vulgaris, L. CV. USH 20) plants were grown two per pot in an environmental chamber programmed to provide a 12-hr light period at 25°C and a 12-hr dark period at 17°C. Light intensity was approximately 1,800 ft-c at the level of the leaf surfaces. Five replicates of each treatment (30 pots) were arranged in a randomized block design, grown for 50 days and harvested. Plants were watered every other day with de-ionized water and once a week given

TABLE 1

Physical and Chemical Characteristics of Fox Sandy Loam and Cd-Enriched Sewage Sludge.

Characteristic	Fox sandy loam	Cd-enriched sludge	Sludge-amended Fox sandy loam
Sand	63%		
Silt	14%		
Clay	21%		
Organic carbon	2.0%	20.95%	
Cation exchange capacity	13.4 meq/100 g	28.43 meq/100 g	
pH	6.3	7.4	
Total N	0.13	1.41	
" P	1245 ppm	15540 ppm	
" K		1278 "	
" Ca		95025 "	
" Mg		6506 "	
" Na		425 "	
" Cd	0.2 ppm	1413 "	29.6 ppm
" Cu	16 "	2150 "	64 "
" Ni	13 "	654 "	22 "
" Pb	16 "	3230 "	
" Zn	45 "	11100 "	219 "

50 ml of half-strength Hoaglands solution minus $MnCl_2$, $ZnCl_2$, and $CuCl_2$. Nitrogen was enriched in the nutrient solution by adding 2.5 mM NH_4NO_3/ liter.

Plant Sample Preparation and Analysis

After harvesting, plants from the same pot were combined, rinsed with de-ionized water, divided into roots and shoots, dried at 85°C, weighed and ground in a Wiley Mill to pass through a 40-mesh screen. Weighed portions of plant material were analysed for heavy metal concentrations as reported elsewhere.[12]

RESULTS AND DISCUSSION

Heavy Metal Uptake and Plant Growth

Addition of sludge to soil did not affect Cu, Ni and Pb concentrations in beet shoots across all phosphate treatments (Table 2). A zinc concentration of 209 ppm was found in the shoots of plants grown in soil which was amended with sewage sludge but not phosphate. This was significantly ($P \leq .05$) greater than the Zn concentration of shoots from plants grown in sludge-amended soil to which phosphate was added (see Table 2). Shoot Zn concentration in corn has also been reported to decrease with increasing amounts of phosphate[9]. The high Zn content of the sludge (11,100 ppm) is most likely responsible for the high concentration of Zn in shoots of beets grown in sludge-amended soil. However, the phosphate addition appears to reduce Zn transport in plants. Addition of sludge to soil, in general, increased Cd concentrations in shoots while no effect on shoot Cd content was observed with phosphate treatments. Shoot Cd concentrations for plants grown on sludge-amended soil treated with 261 lbs/acre phosphate did not significantly differ from those of plants grown on soils given no sludge.

Reduced yields and increased Zn, Cu and Cd concentrations in roots of plants grown on sludge-amended soil was observed (Table 3). The concentration of Pb in roots of plants grown on soils to which sludge was added were in general greater

TABLE 2

Effects of Phosphate Treatments on Yield and Heavy Metal Concentrations of Sugar Beet Shoots Grown of Fox Sandy Loam Amended with Sludge (n=5)

	Sludge treatment (t/acre)					
	0			8.85		
	Phosphate treatment (lbs/acre)					
	0	261	522	0	261	522
Yield (g/pot)	0.663^{a*}	0.577^{a}	0.579^{a}	0.491^{a}	0.421^{a}	0.618^{a}
Zn (ug/g dry weight)	29^{b}	27^{ac}	24^{ac}	209^{d}	47^{bc}	64^{bc}
Cu "	10.4^{a}	11.7^{b}	11.4^{ab}	16.0^{ab}	5.4^{ab}	11.6^{ab}
Ni "	3.2^{a}	3.2^{a}	3.0^{a}	5.4^{ab}	4.6^{ab}	5.3^{b}
Cd "	0.1^{a}	0.2^{a}	0.1^{a}	8.5^{b}	4.4^{ab}	8.2^{b}
Pb "	9.1^{a}	8.1^{a}	7.9^{a}	9.8^{a}	7.1^{a}	7.6^{a}

*Mean values, within rows, followed by the same letter (a-d) are not significantly different at the 5% level according to Duncan Multiple Range Test.

than those of plants grown in soil without sludge. Reduction of sugar beet yields for plants grown on Zn- and Cu-enriched sludge-amended soils concomitant with increased metal concentrations in crops has been reported[13]. Kirkham[6] reported that roots of corn grown in Warsaw silt loam which received sludge applications for 35 years had greater Cd, Cu, Pb and Zn concentrations than those of plants grown on Warsaw silt loam.

Increased Cd concentrations in the above ground portion of soybean grown in soils amended with Cd-enriched sludge was reported[2]. A negative correlation ($r = -.97$) between net photosynthesis and Cd concentration in sunflower leaves has also been observed[14]. In the present experiment, increased Cd concentrations in shoots (Table 2) of plants grown in sludge-amended soil may have reduced leaf photosynthesis and ultimately root growth.

Varying the amounts of phosphate applied did not influence yield or metal concentrations in roots except for Cd in those plants grown on sludge-amended soil (Table 3). The data suggests that less Cd was accumulated by roots when no phosphate was applied than when 261 or 522 lbs/acre were applied. Increasing root cadmium concentration in subterranean clover with increasing soil applied phosphate has been reported[10]. Miller et al.[15] suggests that high soil phosphate levels possibly provide a readily available counterion that accompanies Cd into plant roots.

Distribution and accumulation of given metals varies for different plant species and plant tissues . In the present study, roots of plants grown on sludge-amended soil accumulated greater Cu, Ni and Pb concentrations than shoots. Plants grown on sludge-amended soil accumulated greater concentrations of Zn, Cu, Cd and Pb in roots than plants grown on unamended soil. Knowledge of the particular tissue of a given crop which preferentially accumulates heavy metals is important in deciding which crops can be grown safely on soils amended with sewage sludge. If other root vegetable crops exhibit a similar pattern of metal

TABLE 3

Effects of Phosphate Treatments on Yield and Heavy Metal Concentrations of Sugar Beet Roots Grown on Fox Sandy Loam Amended with Sludge (n=5)

	Sludge treatment (t/acre)					
	0			8.85		
	Phosphate treatment (lbs/acre)					
	0	261	522	0	261	522
Yield (g/pot)	0.459^{a*}	0.489^{a}	0.457^{a}	0.123^{b}	0.068^{a}	0.095^{b}
Zn (ug/g dry weight)	21^{a}	22^{a}	22^{a}	103^{b}	109^{b}	81^{b}
Cu "	19.5^{a}	18.0^{a}	18.9^{a}	36.5^{b}	49.5^{b}	48.0^{b}
Ni "	11.6^{a}	9.1^{a}	10.9^{a}	38.2^{ab}	22.4^{b}	15.5^{ab}
Cd "	0.3^{a}	0.4^{a}	0.3^{a}	4.4^{b}	16.1^{c}	10.3^{bc}
Pb "	10.7^{a}	11.6^{a}	15.7^{a}	64.3^{ab}	70.9^{b}	55.4^{b}

*Mean values, within rows, followed by the same letter (a-c) are not significantly different at the 5% level according to Duncan Multiple Range Test

accumulation to sugar beet, then growth of these crops on sludge-amended soils would be a potential health hazard by increasing Cd and Pb concentrations in food.

Chemical Extractability of Heavy Metals

Addition of sludge to soil increased amounts of DTPA-extractable Zn, Cu, Ni and Cd both prior to planting and following crop harvest, for all phosphate treatments (Table 4). Gaynor and Halstead[8] reported similar results with the addition of sludge but not phosphate to Fox sandy loam, Granby silt loam and Rideau clay. In all sludge-amended soils they observed greater amounts of DTPA-extractable Zn, Cu, Ni and Cd before and after cropping soils with lettuce than in soils to which no sludge was added.

Growth of sugar beets on sludge-amended soil reduced the amount of extractable Zn concomitant with increasing the amounts of extractable Cu and Cd (Table 4). Gaynor and Halstead[8] reported similar results following three consecutive lettuce crops grown on sludge-amended Fox sandy loam. These results suggest that different metals in sludge-amended soils manifest diverse chemical reactions which results in changing their availability. Crop growth on soils to which no sludge was added only affected extractable Cu by increasing its amount. Treatment of soil with phosphate showed no consistent trends in affecting the amounts of extractable metals from soils.

Correlation coefficients (r) of stepwise multiple regression analysis (across all sludge and phosphate treatments) between extractable soil metals (Zn, Cu, Ni and Cd) and plant dry weight or plant tissue heavy metal concentration are given in Table 5. Shoot Zn concentration is correlated with extractable Zn. There is also observed a negative partial correlation between shoot Zn and extractable Cu. In solution cultures, increasing Cu concentrations was shown to reduce Zn concentrations in wheat tops[16]. In the present study

TABLE 4

DTPA-Extractable Heavy Metals in Soil Amended with Cd-Enriched Sludge Before and After Cropping with Sugar Beets*

DTPA-extractable metals	Sludge treatment (T/A)	Before crop			After crop		
		Phosphate treatment (lbs/A)					
		0	261	522	0	261	522
Zn	0	1.19^{b}	1.01^{a}	1.64^{c}	0.94^{a}	1.11^{b}	0.91^{a}
	8.85	43.84^{d}	40.03^{c}	40.50^{c}	29.31^{b}	27.82^{a}	28.86^{ab}
Cu	0	0.68^{a}	0.64^{a}	0.77^{b}	0.91^{c}	0.89^{c}	0.76^{bc}
	8.85	2.29^{a}	2.59^{b}	2.38^{a}	8.93^{c}	8.74^{c}	10.01^{c}
Ni	0	0.28^{a}	0.36^{ab}	0.40^{b}	$0.2,^{a}$	0.28^{a}	0.29^{a}
	8.85	1.75^{b}	1.94^{b}	1.87^{b}	1.43^{a}	1.79^{b}	2.16^{c}
Cd	0	0.15^{ab}	0.12^{a}	0.19^{b}	0.13^{a}	0.13^{a}	0.11^{a}
	8.85	2.29^{b}	1.88^{a}	2.34^{b}	2.60^{c}	2.35^{bc}	2.72^{c}

*Mean values, for each sludge treatment (rows), followed by the same letter (a-d), did not differ significantly at the 5% level according to Duncan Multiple Range Test.

TABLE 5

Correlation coefficients†, r, of stepwise multiple regression analysis (across all sludge and phosphate treatments) between DTPA-extractable heavy metals and yield (dry wt.) or heavy metal content (μg/g dry wt.) of sugar beet shoots and roots.

Plant yield and heavy metal content	Correlation coefficients, r, of DTPA-extractable soil heavy metals			
	Zn	Cu	Ni	Cd
		Shoots		
Dry weight				
Zn	.597***	(-.739***)#		
Cu				
Ni				.617***
Cd				.765***
Pb				
		Roots		
Dry weight		-.930***		
Zn		.852***		
Cu			.798***	
Ni	.498**			
Cd	(-.518**)#	.727***		
Pb		.660***		

† Correlation coefficients are given only for those values which are significant at the .01 level or greater.

*** $P \leq .001$.

** $P \leq .01$.

Partial correlation coefficients that account for a significant ($P \leq .01$) amount of variation.

the Cd and Ni content of shoots are correlated with extractable Cd. Others have also demonstrated significant correlations between extractable (0.1 N HCl) Cd and shoot Cd of plants grown on sludge-amended soils[2].

Root dry weight has a strong negative correlation with extractable Cu (Table 5). Hemphill[17] states that plants normally contain 5-20 ppm (on a dry weight basis) Cu and that toxicity symptoms appear with Cu levels greater than 20 ppm. In the present study, shoots of all plants and roots of plants grown on Fox sandy loam have Cu concentrations between 5.4 and 19.5 ppm (Tables 2 and 3). Roots of plants grown on sludge-amended soil contain from 36.5 to 49.5 ppm Cu, and show a significant yield reduction. In soils enriched with Cu (as $Cu(OH)_2$ and $CuSO_4$), negative correlations between DTPA-extractable soil Cu and snapbean yield was observed by Walsh et al[18]. Whereas they reported that a significant yield depression of snapbean occurred when extractable soil Cu exceeded 20 ppm. In the present study, significant yield reduction of beet roots occurred with extractable Cu concentrations in soil between 8.7 and 10 ppm. These results show that plant species differ in their abilities to accumulate heavy metals from soils.

Cadmium uptake by roots shows a negative partial correlation with extractable Zn. These results differ from those obtained by Haghiri[19] who reported that Cd concentrations in oats increased with increasing amounts of soil applied Zn (as $Zn(NO_3)_2$) from 0-50 ppm. Comparison of the observations made by Haghiri[22] with those of the present study again may reflect the differences in behavior of plant species with respect to elemental uptake.

Extractable Cu in the soil appears to be the most important metal with respect to affecting roots of plants grown on sludge-amended soil. A possible synergistic effect of extractable Cu on the uptake of Zn, Cd and Pb by beet roots is observed. If increasing the amount of available soil Cu stimulates Zn, Cd and Pb uptake by plant roots, then addition of sludges containing

relatively small concentrations of Zn, Cd and Pb but large concentrations of Cu to soils in which root vegetables are to be grown, would increase potential health hazards by increasing Zn, Cd and Pb concentrations in food.

SUMMARY

The present series of experiments demonstrates that Zn, Cu, Cd and Pb accumulation by sugar beets increases when soil is amended with sludge. The metal concentrations vary according to plant tissue. Roots accumulate greater Cu, Ni, Cd and Pb concentrations than shoots. Phosphate treatment does not affect the accumulation of heavy metals by plants.

Addition of sludge to soil increased DTPA-extractable Zn, Cu, Ni and Cu from soils. Cropping of sludge-amended soil reduced the amount of extractable Zn while increasing amounts of extractable Cu and Cd. Phosphate treatment does not affect the amounts of extractable metals from soils.

Stepwise multiple regression analysis between extractable heavy metals (Zn, Cu, Ni and Cd) and dry weight or tissue heavy metal concentrations shows Cu to have most influence on growth and heavy metal uptake by beet roots. Beet yield is negatively correlated with extractable Cu and beet root yield reduction of plants grown on sludge-amended soil is attributed to Cu toxicity. Positive correlations, suggesting synergisms, between extractable Cu and Zn, Cd and Pb uptake by beet roots were observed. In light of these possible synergisms, addition of sludge with low Zn, Cd and Pb concentrations but high Cu concentration to soils cropped with root vegetables may increase potential health hazards by increasing the amounts of these heavy metals in the human food chain.

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THE USE OF ZONAL CENTRIFUGATION IN DELINEATING TRACE ELEMENT DISTRIBUTIONS IN SEWAGE SLUDGE AND SOIL

by

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ABSTRACT

Isopycnic zonal centrifugation, using tetrabromoethane-ethanol density gradients, was used to fractionate sewage sludge and soil. Up to 75 weight percent of sludge has relatively low density (1.60-2.10 gm/cm^3). Eighty weight percent of soil has relatively high density (2.50-2.90 gm/cm^3). Organic matter makes up a large portion of low density material.

Trace concentrations of cadmium, copper, lead, nickel, and zinc in sludge and soil fractions are distributed in a regular fashion with respect to density. Seventy-five weight percent of the total trace elements in sludge is contained in organic-rich fractions. In soil, there is a strong positive correlation between trace element concentrations and organic matter content. A relatively small amount of the trace elements in sludge appears to be associated with minerals, particularly sulfides and sulfates, in high density fractions.

Since a high weight percentage of the trace elements in sludge and soil is associated with organic matter, it is concluded that most of the trace heavy metals are in organic compounds. It appears that particular organic compounds, within a limited density range, contain the greatest concentrations of trace elements. Infrared spectroscopy suggest carboxyl group compounds as the most likely hosts for the trace heavy metals.

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INTRODUCTION

The growing trend to recycle sewage sludge as a soil conditioner and fertilizer on agricultural lands requires a careful analysis of its effects on the soil-groundwater-plant system. Although sewage sludge has many beneficial qualities and essential nutrients, it often contains hazardous materials. Perhaps the greatest danger of amending soil with sludge involves the harmful effects of heavy metals such as cadmium, lead and zinc on the environment. These trace elements may enter the food chain and have been shown to be toxic to plants and man (Chaney, 1973). Many workers have shown that a considerable portion of the total trace elements in sludge is available for translocation in plant tissues and the soil system (LeRiche, 1968; Anderson and Nilsson, 1972). The interaction of sewage sludge with the ecosystem is highly variable and depends on such soil and sludge characteristics as pH, CEC, EA, major and minor element concentrations, and field conditions (Page, 1974).

An additional important aspect of sludge is the molecular species of contained trace heavy metals. The chemical form of trace elements plays an important role in determining the types of trace element transformations in the soil system. Page (1974) believes that organically bound trace elements probably undergo microbial and chemical transformations once sludge is applied to soil, while a large portion of an inorganic form remains stable and unchanged. Miller and Zaebst (1972) found that sludge organic matter rapidly oxidizes when applied to soil, resulting in the release of many organically held trace elements. Therefore, an understanding of the chemical speciation of trace elements in sewage sludge would be a valuable asset in making decisions regarding land application of sludge. Also, once the compounds are known, reversion methods which render trace elements immobile might be developed to transform elements into chemical forms less

available to plants and the soil system. Chaney (1973) has shown that reversion is most rapid in soils containing high carbonate concentrations, and is related to soil pH, PO_4, and organic matter. The process of extracting trace metals from sludge as a resource might also benefit from the knowledge of their molecular forms.

It is very difficult to determine the chemical compounds which contain trace elements in sludge, due to its great chemical and physical complexity. Cothern et al. (1977), using electron spectroscopy for chemical analysis (ESCA), indicate that zinc and copper are bound in a molecule containing oxygen, either organically (possibly carboxylate) or inorganically (oxide, nitrate, sulfate, phosphate, etc.). Other workers have used infrared spectroscopy in dealing with this problem of speciation, and their results suggest the carboxyl group as a likely molecular species of trace elements (Tan, King, and Morris, 1971). The wide number of origins and unique chemical environments encountered by sewage sludge results in a material highly complex and varied. It would be desirable, therefore, to somehow separate sludge into components with the hope of removing this complexity. A mechanical technique of separation, zonal centrifugation, is believed by the authors to be a valuable process in performing very detailed density separations on sludge.

Zonal centrifugation, a relatively new technique, received its initial use in biological cell separation work. Francis et al. (1976) were the first to use the technique in soil science and were successful in performing very detailed density, hence mineralogical, separations on soils and sediments. Zonal centrifugation involves an isopycnic separation (based on a particle's buoyant density) in a density gradient liquid of a desired density range. Brantley et al. (1974), in an extensive search, showed that tetrabromoethane (TBE) solutions with ethanol (ETOH) as a diluent were the best density gradient liquids for use in separation of soils.

With the purpose of delineating trace element and mineralogic trends in

sewage sludge and soil, zonal centrifugation was employed in the present study for separation of these materials. This paper describes the methodology used and discusses the mineralogical compositions and trace element distributions of selected sludges and soils.

MATERIALS AND METHODS

Sample Preparation

Two sewage sludges and one soil were selected for separation: the Dayton, Ohio, sewage sludge, a composite of both domestic and industrial wastes, the Englewood, Ohio, sewage sludge, composed primarily of domestic waste, and the Miami silt loam (MSL), a Montgomery County, Ohio, upland till, agricultural soil. The MSL was collected from the upper 12" at a location in the SE¼, SW¼, Sec. 13, T.6N., R.5E., of Montgomery County, Ohio, homogenized in the lab, and sieved to a particle size (p.s.) of <44 microns. Anaerobically digested liquid Englewood, Ohio, sludge (1 wt. % solid) was dried at 110°C. The Dayton sludge received different preparation. Primary (undigested) liquid Dayton sludge was spiked with approximately .01 weight percent (wt. %) cadmium (as $CdNO_3$) and anaerobically digested according to Brown, 1977, with the hope of incorporating added cadmium into the sludge in its predominant molecular species. Once digestion was completed, the liquid sludge (3 wt. % solid) was dried at 110°C. Many workers have added salts of trace elements to sludges under investigation (Bingham et al., 1975; Jones et al., 1973; Stenström, 1974). However, to our knowledge, none have attempted to add them prior to anaerobic digestion, and therefore none have incorporated these trace elements in their proper chemical forms. Anaerobically digested Dayton sludge obtained from the drying beds of the Dayton, Ohio, Wastewater Treatment Plant was also run for comparison. All dried sludges were crushed with an agate mortar and pestle, homogenized, and sieved to a p.s.<44 microns. For a listing of the physical and chemical characteristics of the

sludges and soil, see Table 1.

Zonal Centrifugation Technique Employed

A Beckman model J-21B Supercentrifuge with a model JCF-Z titanium alloy large scale zonal rotor (including Ti core) was used in all separations. The rotor is equipped with a seal assembly that allows unloading and loading of density gradient liquids while the rotor is spinning. This permits relatively intact transferal of any desired type of density gradient (stepwise, linear, concave, convex, etc.). Since a density gradient liquid such as TBE-ETOH is involved, conventional "O" rings of Neoprene would be easily destroyed. This requires the use of Viton "O" rings, which do not react with TBE. The gradients were made by using a gradient former similar to that developed by Anderson and Rutenburg (1967) (See Fig. 1). All density gradients were made using varying concentrations of pure TBE (density - 2.94 gm/cm^3) and 10% Polyvinylpyrrolidone (K-30) in ethanol (density - 0.82 gm/cm^3). K-30 is used to maintain dispersion of the sample particles in the gradient liquid. The procedure was adopted from that of Francis et al. (1976) as follows (refer to Fig. 1 for equipment):

With the rotor spinning at 2000 rpm and at 25°C, 100 ml each of 1.3 gm/cm^3 and 1.6 gm/cm^3 gradients are loaded into the rotor. These act as low density "cushions." Then, with the gradient former filled with 1.8 gm/cm^3 gradient, 1300 ml of 2.75 gm/cm^3 gradient is gravity fed into the mixing bowl while the circulating pump is operating to mix the gradient. A peristaltic pump simultaneously pumps the forming gradient into the rotor. These gradients are sequentially added to the edge of the rotor, continuously forcing gradients of lesser density toward the center. After this step is completed, the rotor contains an approximately linear density gradient ranging from 1.3 gm/cm^3 to 2.75 gm/cm^3. The oven-dried sample (10-15 grams) is added to 100 ml of 10% K-30 in ethanol and dispersed for four minutes using a Bronson model S-75 ultrasonic

TABLE 1. PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE SLUDGES AND SOIL FRACTIONATED

Heavy Metal Concentration (μg/g dry wt.)

Mean ± S.D. (n=3)

METAL	DAYTON SLUDGE*	ENGLEWOOD SLUDGE	MIAMI SILT LOAM
Cd	414 ± 11	3.5 ± 0.3	0.15 ± .006
Pb	3230 ± 65	240 ± 25	24.7 ± 1.0
Ni	654 ± 6	1325 ± 45	18 ± 2
Cu	2150 ± 78	950 ± 25	10.3 ± 0.6
Zn	11100 ± 250	915 ± 5	58.8 ± 0.4
	Other Elements (% dry wt.)		
Na	.04	.16	.01
N	1.40	2.50	0.21
K	1.28	3.73	.40
P	1.55	1.75	.12
Ca	9.50	5.83	1.06
Mg	.91	1.02	.29
Organic carbon (%) n=3	21.0 ± 1.0	44.5 ± 1.8	5.7 ± 0.2
% Volatiles (n=3)	53.5 ± 0.3	53.1 ± 0.4	4.8 ± .04
pH (n=3)	6.34 ± 0.1	6.29 ± .08	5.35 ± .1
CEC (meq/100 gm)	28.4 ± 2.8	52.3 ± 5.0	22.5 ± 1.8
EA (meq/100 gm)	20.8 ± 2.7	26.1 ± 1.7	8.1 ± 0.9

* Heavy metal concentrations of the lab-digested Cd-spiked Dayton Sludge are the same as that of the drying bed Dayton Sludge except Cd = 1413 ± 12 μg/gm for the former.

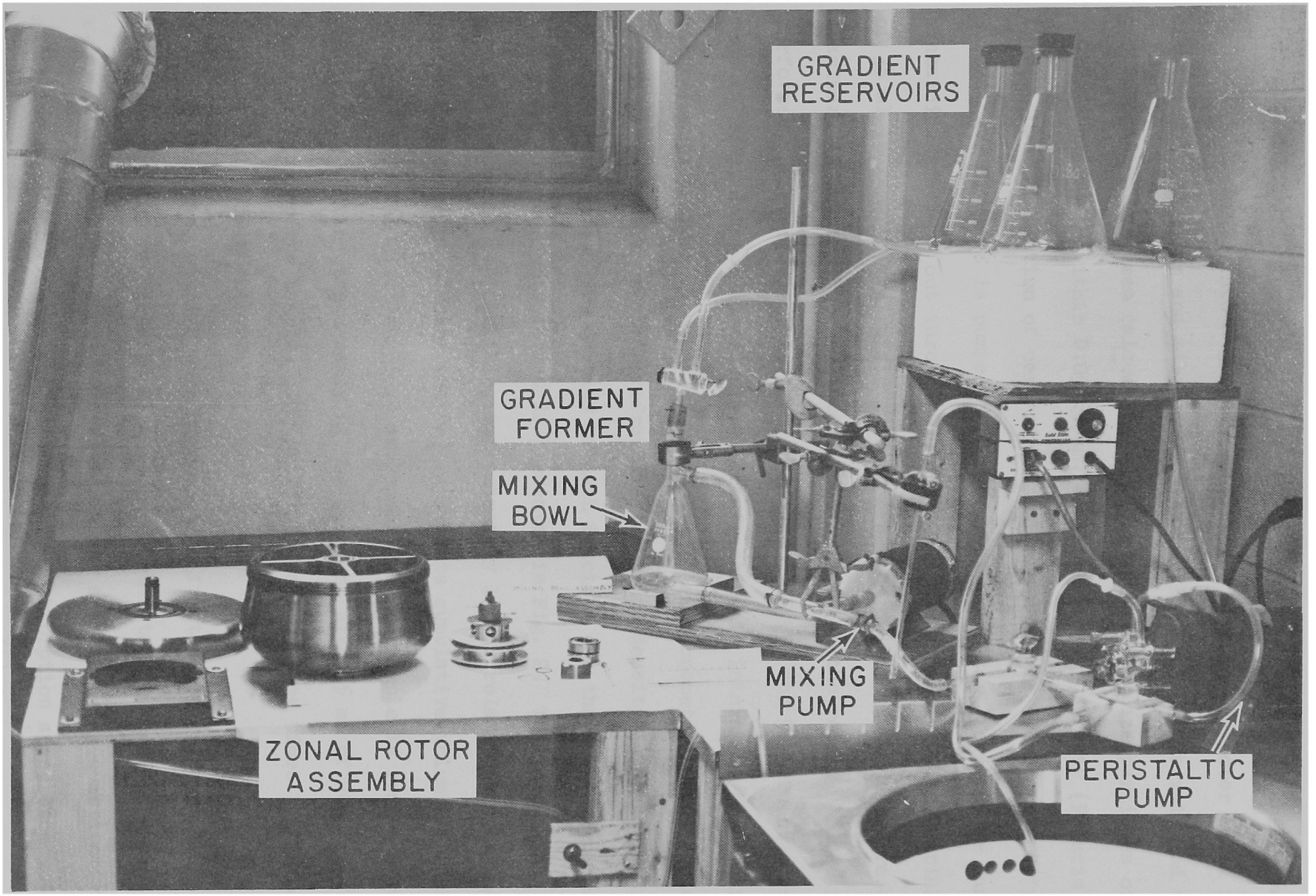

Figure 1. Zonal rotor assembly and density gradient forming equipment used in the separation of sewage sludge and soil by super centrifugation.

probe, just prior to loading. The sample is then pumped into the center of the rotor followed by approximately 350 ml of 2.75 gm/cm^3 gradient ("overlay") to place the sample into a greater centrifugal field. The rotor speed is increased to 13,000 rpm and allowed to spin for 25 hours. This rate and time of centrifugation has been calculated to bring .05 micron-size particles of density 2.0-2.75 gm/cm^3 to within 0.05 gm/cm^3 of their buoyant density (Francis et al., 1976). Upon completion of centrifugation, the rotor is slowed to 2000 rpm for unloading. Pure TBE is pumped to the edge of the rotor, forcing the sample-containing gradient out through the center. Approximately 55 sequential fractions of 35 ml increments are retrieved in a fraction collector. They represent imaginary adjacent hollow cylinders that existed in the rotor. In the four runs performed, recovery was very high, namely 85, 95, 91 and 96 percent recovery for runs 1, 2, 3, and 4 respectively. Each fraction is internally homogenized and the refractive index is measured. Using a 10 ml pycnometer and a refractometer, a calibration curve relating refractive index and density is developed for TBE-ETOH gradients. The density of each fraction can thereby be deduced. The nature of the gradients formed is presented in Fig. 2. This graphical representation shows a reproducible, approximately linear relationship between rotor volume and density. "Rotor volume" represents cumulative volumes of each sequential fraction (i.e. in 35 ml increments). The sample is removed from its gradient by cutting the density of the gradient with 15 ml of 200 proof ETOH and centrifuging the sample. The pellet is washed three times with 50 ml ETOH or until all TBE is removed.

The following studies were conducted on fractionated sludges and soil: (1) determination of percent volatiles, (2) qualitative chemical analysis by x-ray fluoresence and energy dispersive x-ray analysis, (3) quantitative trace and selected major element analysis by atomic absorption spectroscopy, (4) organic compound identification by infrared spectroscopy, (5) inorganic compound (mineral)

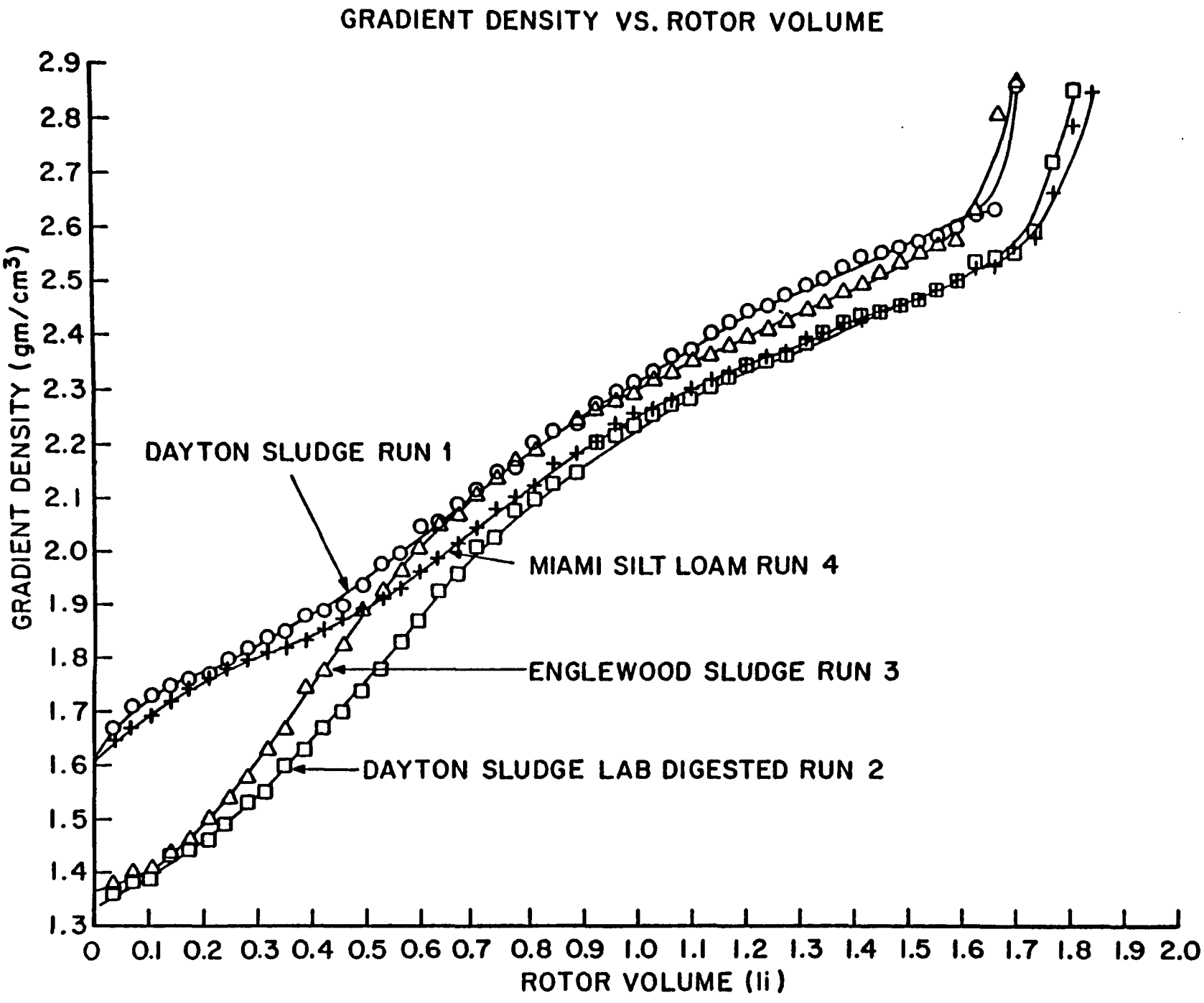

Figure 2. Plot showing distribution of densities in gradient fluid for sewage sludges and soil.

identification by x-ray diffraction analysis and (6) optical analysis using a polarizing petrographic microscope.

RESULTS AND DISCUSSION

Distribution of Density Fractions

A plot was made of the weight percentage of the total sample accounted for by each density fraction versus the density of the fraction (Fig. 3). The distributions for the two sludges and the soil are fairly simple. Each sludge or soil contains from one to three peak weight percentages. The Dayton sludge-drying bed (DSDB) sample distribution shows a primary peak centered at 2.03 gm/cm^3. Approximately 40 wt. % of the total sample occurs within the density range 1.95-2.15 gm/cm^3. There is a secondary peak at 2.85 gm/cm^3, and about 10 wt. % of the total sample occurs from 2.7-2.9 gm/cm^3. Lab digested Dayton sludge (DSLD) has a primary peak centered at 1.97 gm/cm^3. Sixty-three wt. % of the total sample occurs within the range of 1.9-2.1 gm/cm^3. A secondary peak is at 2.85 gm/cm^3, and about 5 wt. % of the total sample occurs from 2.7 to 2.9 gm/cm^3. While the positions for the peaks of the two Dayton sludges are almost the same, the DSLD sample contains more material in the lower-density range than does the DSDB sample. This may be due to the inclusion in the latter sludge of sand and other material from the drying bed. The Englewood sludge has three peaks: a primary peak centered at 1.73 gm/cm^3, with about 75 wt. % of the total sample within the range 1.6-1.9 gm/cm^3; and two secondary peaks centered at 2.13 and 2.65 gm/cm^3 with about 7 and 4 wt. % respectively. The difference in the Englewood sludge may be due to the fact that it is almost completely domestic in origin. The Miami silt loam soil has one peak centered at 2.75 gm/cm^3 which contains about 80 wt. % of the total sample within the range 2.5-2.9 gm/cm^3.

Determination of Percent Volatiles

During the processing of samples for trace element analysis, they were

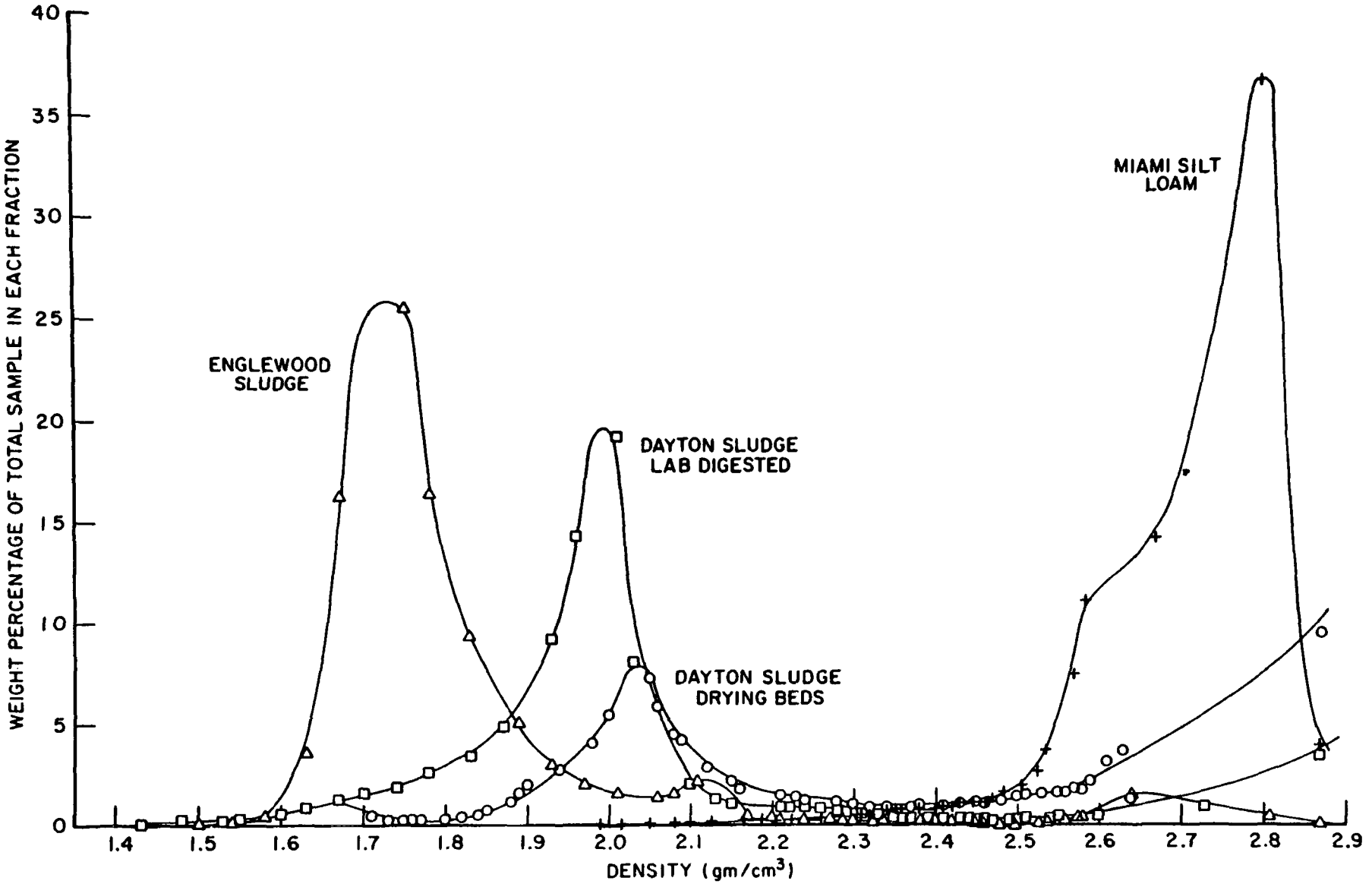

Figure 3. Plot showing distribution of centrifuge fractions of sewage sludge and soil according to density and weight percentage of total sample in each fraction.

ashed at 500 C.° The samples were weighed before and after ashing and percent volatiles was determined. The samples were oven-dried at 110 °C before ashing to drive off contained surface water. The ashing temperature was not high enough to destroy any of the inorganic material, such as driving the carbon dioxide out of the carbonates (~900 °C). It is believed that the loss of weight is due mainly to the disintegration of organic material. Therefore, percent volatiles can be used as an approximation of the percent organic matter (EPA, 1971). Figure 4 is a plot of percent volatiles versus density. The trends of the three samples are remarkably similar. The two sludges decrease from 95% volatiles in the very low density fractions (1.4-1.5 gm/cm^3) to less than 10% volatiles in the high density fractions (~2.8 gm/cm^3). The soil contains 45% volatiles in the 1.60 gm/cm^3 fraction. The volatiles decrease to 2.9% in the 2.87 gm/cm^3 fraction. It appears that the percent volatiles and thus, the percent organic matter in both sludges and the soil decreases linearly with density up to about 2.1 gm/cm^3 and decreases exponentially with greater densities. The low density material of the primary peak in the DSDB contains about 35 wt. % of volatiles, whereas the material of the primary peak in Englewood sludge contains about 70% volatiles.

Inspection of fractions under a microscope showed that many particles of sludge and soil are aggregates of crystalline and organic matter. We did not get perfectly clean separation of organic and inorganic material. Perhaps the samples were not fine enough or there was insufficient dispersal with the ultrasonic probe.

Qualitative Chemical Analysis

Table 1 shows a partial chemical analysis of Dayton and Englewood sludges and Miami silt loam soil. Qualitative x-ray fluoresence and energy dispersive x-ray analysis of whole sludge and density fractions shows that iron, sulfur,

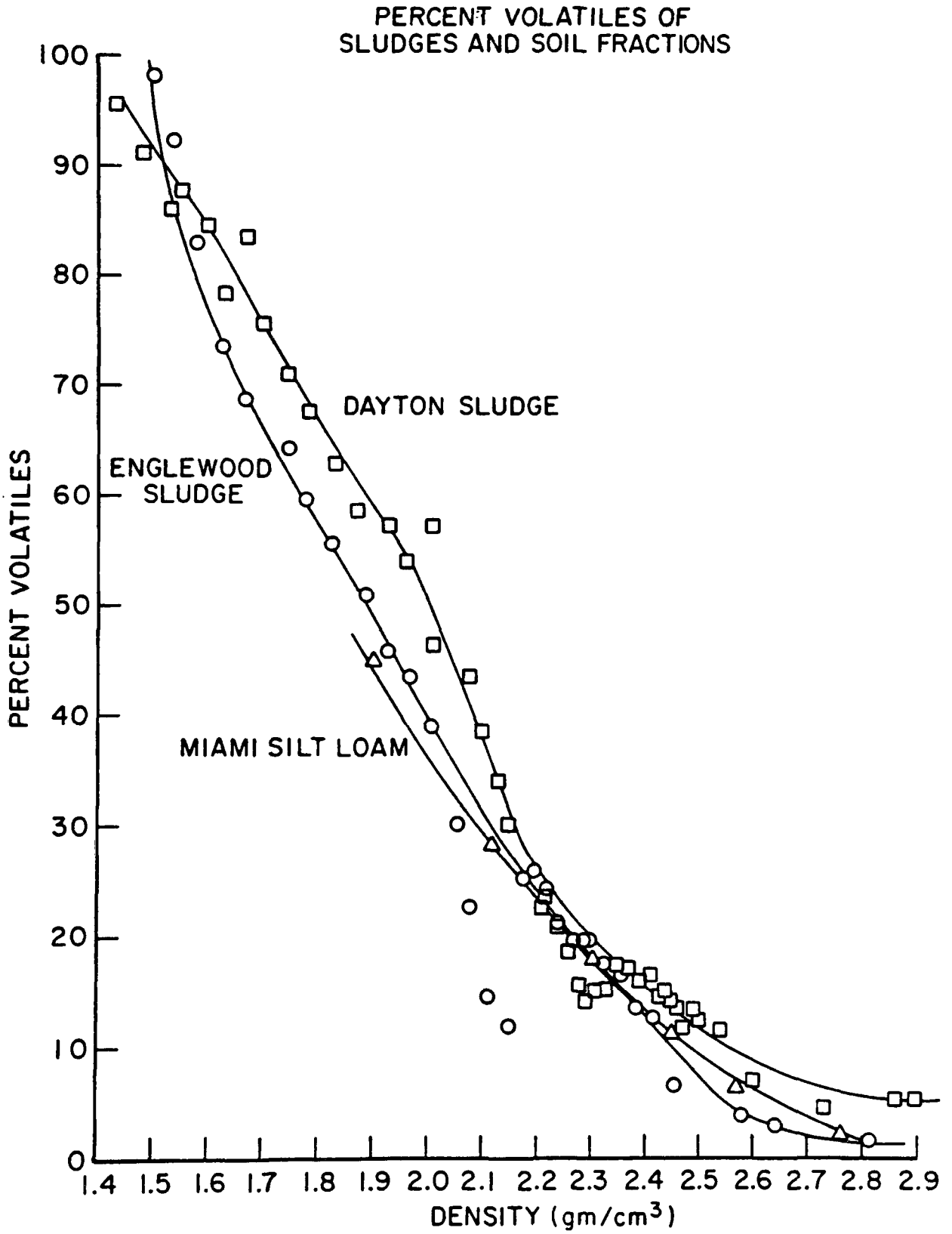

Figure 4. Plot showing density of sewage sludge and soil density fractions versus percent (wt. %) volatiles in the fractions.

titanium, silicon, aluminum, chlorine and chromium are also present.

Trace Element Distributions

Distributions of cadmium, copper, lead, nickel and zinc with respect to density in the Dayton sludges, Englewood sludge, and Miami silt loam are presented in Figs. 5-8. All distributions show curve-like, continuous trends, implying that more than random scattering accounts for the distributions. The two Dayton sludges show similar trends for all five elements (Figs. 5 and 6). Concentrations of cadmium, copper, lead, and zinc increase with increasing density until they reach a maximum at 2.05 gm/cm^3. They then decrease until reaching a minimum at 2.6 gm/cm^3. Nickel, however, tends to decrease from a very low density maximum to a minimum at 2.6 gm/cm^3. All elements measured trend toward a secondary maximum in the very high density range (2.70-2.89 gm/cm^3). Englewood trace element distributions are different from those for Dayton sludge (Fig. 7). Two similar maximum concentrations of all five elements exist at the very low (1.40-1.50 gm/cm^3) and very high (2.70-2.90 gm/cm^3) density ranges. Intermediate densities show a general trend to slowly decrease, with nickel, copper, and zinc drastically minimizing at $\sim$2.15 gm/cm^3 where concentrations are one-tenth of the maximum. The difference between Dayton sludge and Englewood sludge is that in the latter, the low density maximum trace element concentration is at 1.4 gm/cm^3. This is significantly less than the maximum concentration in Dayton sludge at about 2.00 gm/cm^3. Again, the predominantly domestic nature of the Englewood sludge may be the reason for this. The trend of trace metals in the Miami silt loam soil is seen to decrease linearly as density increases for all elements except lead (note logarithmic plot in Fig. 8).

It is noticed that two different narrow density ranges in the Dayton sludge contain the greatest concentrations of trace elements: the low density range (40% volatiles) centered at 2.05 gm/cm^3; and the secondary range (10% volatiles) centered at 2.85 gm/cm^3. When considering absolute amounts of trace elements, however, the organic-rich primary range contains nearly 75 wt. % of the total

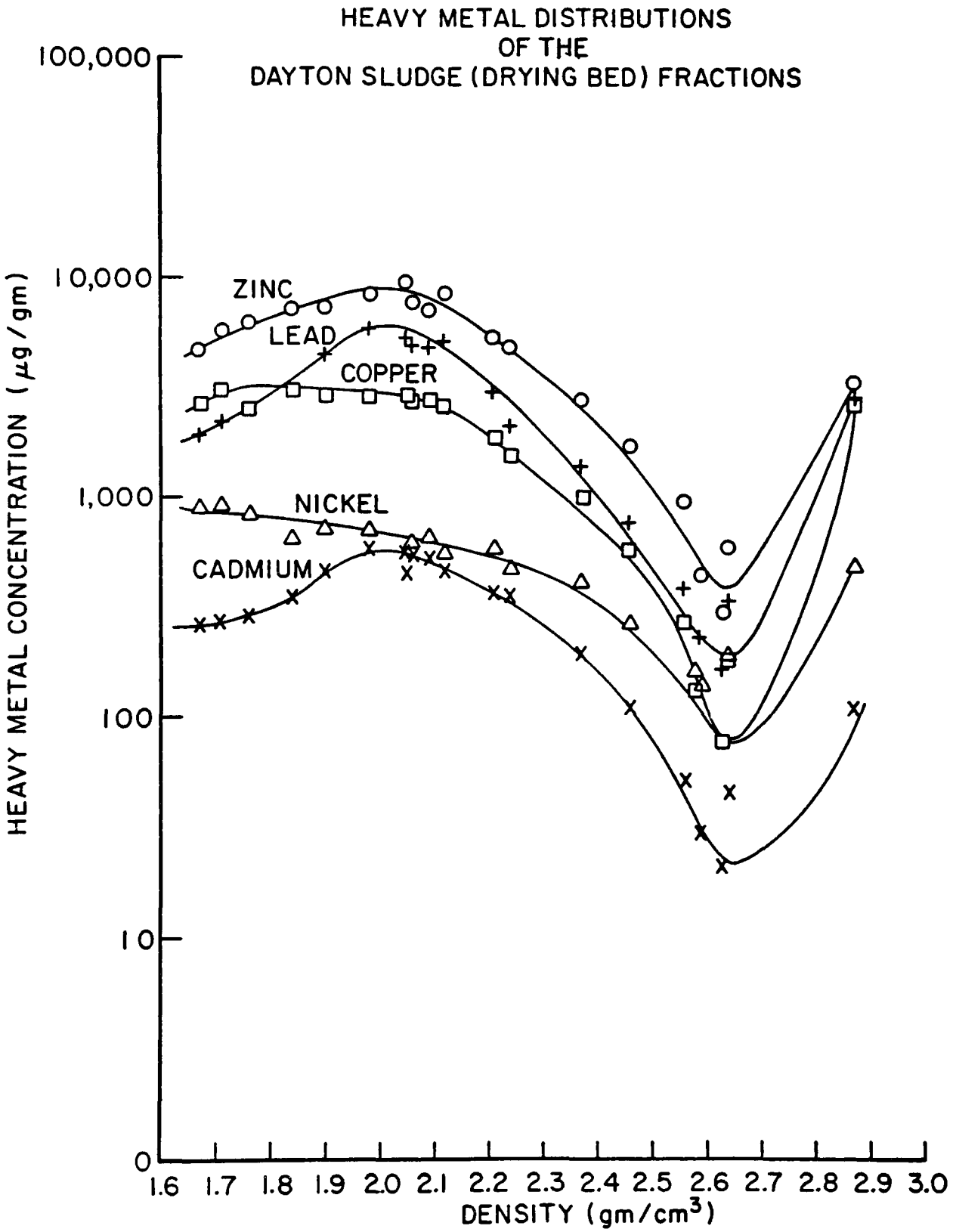

Figure 5. Plot showing distribution of trace elements with respect to density in Dayton sewage sludge from drying-bed.

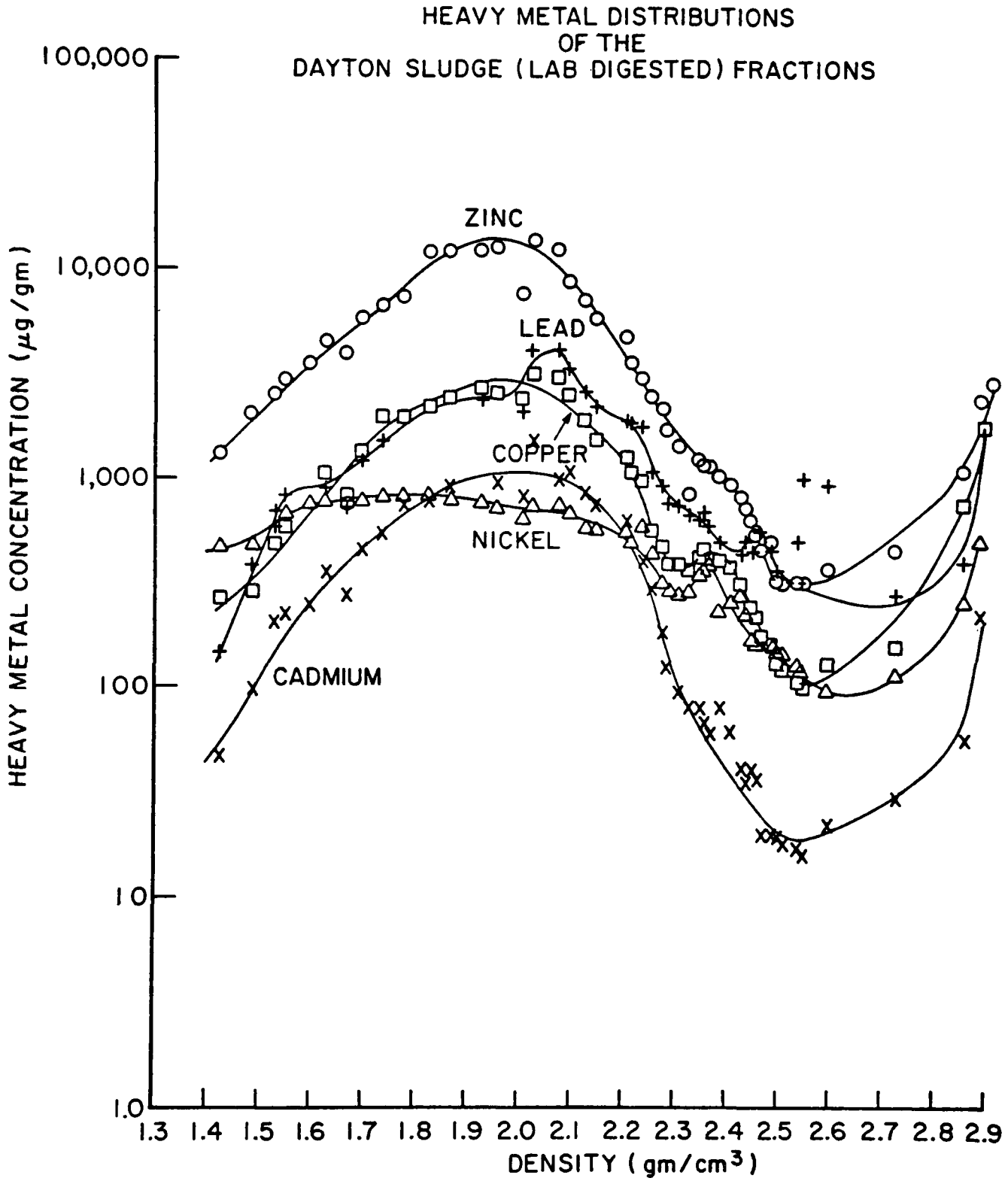

Figure 6. Plot showing distribution of trace elements with respect to density in Dayton sewage sludge digested in the lab.

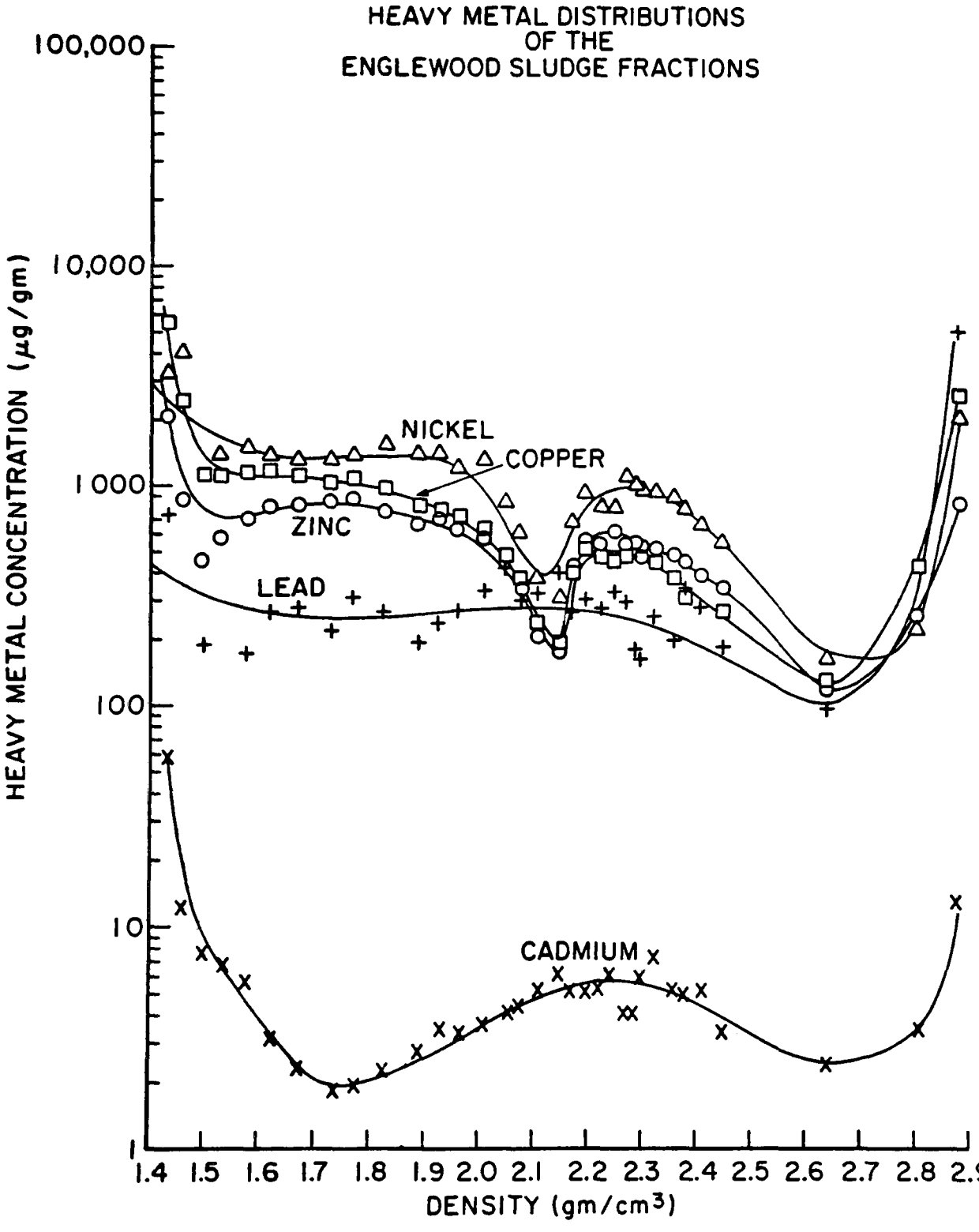

Figure 7. Plot showing distribution of trace elements with respect to density in Englewood sewage sludge.

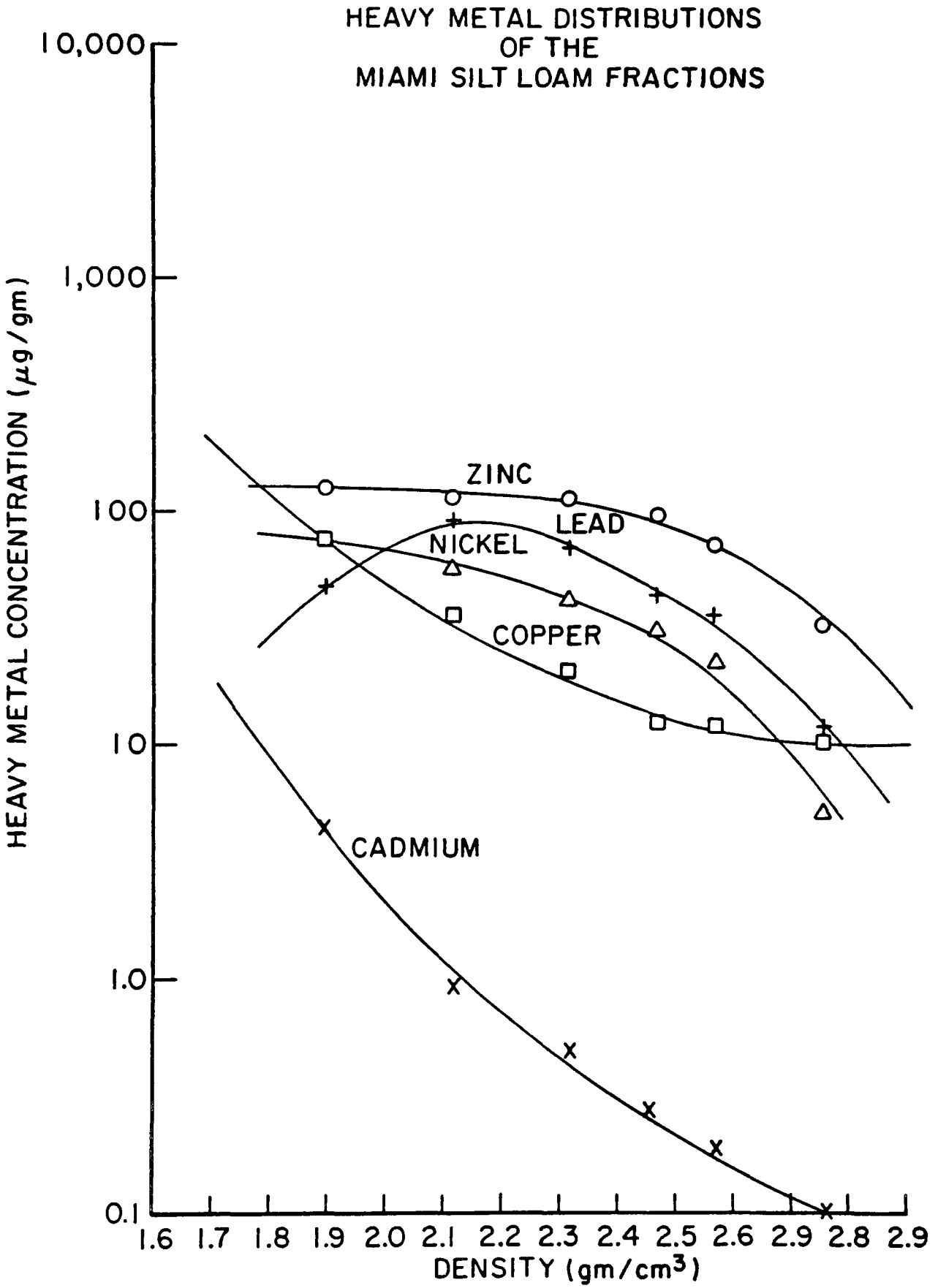

Figure 8. Plot showing distribution of trace elements with respect to density in Miami silt loam soil.

trace elements. It is important to note that with the Dayton sludge, the high trace element concentrations are in the density range which includes the greater part of the total weight of the sludge sample. In the Englewood sludge, maximum trace element concentrations are in a lower density range. Seventy-five weight percent of the total trace elements in Englewood sludge are present in the organic-rich primary range. Correlation of high trace element concentrations with both high and low organic matter content points toward a dual mechanism in molecular speciation - organic and inorganic. Since 75 wt. % of the trace elements in both sludges are in high organic matter fractions, it appears that organic forms predominate.

A remarkable positive correlation exists between trace element concentration and percent volatile material in the Miami silt loam soil. Simple correlation analysis yielded the Pearson correlation coefficients seen in Table 2. All elements except Pb significantly correlate with % volatiles. This implies that in the Miami silt loam, organic forms of trace elements predominate.

Table 2. Pearson simple correlation coefficients between percent volatile matter and trace element concentrations, in density fractions of Miami silt loam.

	Trace Elements				
	Cd	Cu	Pb	Ni	Zn
% volatiles	0.90	0.96	0.54	0.98	0.82
Level of significance	0.007	0.001	0.136	0.001	0.024

Brantley et al. (1973) found that the TBE-ETOH gradients extracted up to 68% of Cd^{109} that had been added to soil. We estimated the amount of extraction of the heavy metals by: (1) adding up the absolute amounts of the various elements in the fractions of Dayton sludge and determining what percentage this was of the absolute amount of a given element in a whole sludge sample of equal weight; and (2) subtracting this amount from 100%. Table 3 shows the percent of extraction (loss) for each metal. The amount of cadmium lost (25%) is much less than the

loss reported by Brantley et al. They simply added cadmium to soil. Since we added cadmium to sludge while it was being digested, it is believed that it was more securely bound than if it had been added after digestion. Although extraction was up to 29% (lead), we do not believe that this greatly affected the trace element distribution pattern described above.

Table 3. Percent of total heavy metals extracted by TBE-ETOH from Dayton sludge.

Heavy metal	% of heavy metals extracted
Lead	29
Cadmium	25
Zinc	21
Nickel	1.0
Copper	0.0

Analysis of Sewage Sludge by Infrared Spectroscopy

Whole and fractionated sludge samples with high trace element values were examined by infrared spectroscopy. Cothern et al. (1977) report that copper and zinc in Dayton sludge are bonded to oxygen and are probably in similar compounds. Since cadmium forms compounds similar to those of zinc, it appears that it is also bonded to oxygen. The most likely organic metal complexing compounds are probably the carboxyl group. Tan et al. (1971) extracted organic matter from sewage sludge. This material was separated into high and low molecular weight fulvic fractions. These fractions were mixed with 0.1 N $ZnCl_2$. The zinc had greater affinity for the lower molecular weight fraction. Using the low molecular weight complex, Tan et al., found that "the organic preparations had infrared characteristics similar to those reported earlier for polysaccharides. A shift in OH bond from 3500 to 3200 cm^{-1} and a sharp increase in carboxyl stretching vibration at 1650 and 1400 cm^{-1}, observed in the spectrograms of the zinc complexes,

indicated the formation of coordinate covalent bonds between OH groups and zinc, and electrovalent linkages between $(COO)^{-1}$ and zinc respectively."

In the present study, sewage sludge samples were leached with aqueous base and the insoluble solids removed by filtration. Extraction of the basic filtrate with diethyl ether removed water-soluble, non-ionic organic bases and neutral materials. Acidification of the aqueous layer with mineral acid followed by extraction with diethyl ether yielded an extract containing weakly acidic materials such as carboxylic acids. The infrared spectra suggest the presence of more than one type of carboxylate anion in this extract. Although the spectra are somewhat similar to those obtained by Tan et al., no metal compound identification could be made.

X-Ray Diffraction and Microscopic Analysis of Sewage Sludge and Soil

About 40 x-ray powder camera and diffractometer patterns were run on whole and fractionated samples of sludge and soil. Sharp diffractometer peaks from crystalline material are present along with broad peaks from amorphous organic material.

X-ray and microscopic investigation of sludge

Under the microscope, the organic matter in the sludge appears to have three occurrences. First, it appears in brown to black opaque masses which make up from 30-40 volume percent (vol. %) of the low density material, and it decreases to 5-10 vol. % of the high density material (Table 4). Second, the organic matter appears in light to dark brown masses which make up from 60-70 vol. % in the low density fractions to none in the high density fractions. This material has a very intricate, multicolored, curved line pattern with the upper polar out. Under crossed polars, some of the matter in these masses is birefringent, indicating the presence of crystalline material. Since these masses occur, in places, on the surfaces of feldspar crystals, it is believed that they are a mixture of

TABLE 4. DENSITY - COMPOSITION OF SELECTED DAYTON SEWAGE SLUDGE CENTRIFUGE FRACTIONS

Density (gm/cm^3) Fraction	Wt. % of Total Sample	Wt. % Volatiles	Volume % Organic Matter	Volume % Quartz & Feldspar	Volume % Carbonates	Volume % Other Minerals	Other Minerals
1.75	.35	96	99	< 1.0	< 1.0	0	------
2.01	19.30	57	90+	1-3	1-3	1-3	Amesite Corundum
2.05	7.4	45	80+	1-5	1-5	1-5	Borickite
2.35	.40	17	30-40	50	< 10	< 10	Amesite, Borickite, Boracite, Gypsum-anhydrite, Garnet, Corundum, Chalco-pyrite, Illite
2.61	3.18	7.0	5-10	60-70	10-15	< 5	Illite, Magnetite, Corundum, Garnet, Hematite, Boracite, Pyrite, Chalcopyrite
2.89	.02	5.2	< 10	20	65	5-10	Magnetite, Corundum, Garnet, Hematite, Boracite, Gypsum-anhydrite, Amesite, Goethite, Pyrite, Chalcopyrite, Sphalerite

illite with organic matter and possibly goethite in places. Amesite, a member of the micaceous chlorite family, may also be present. The third occurrence of organic material is as plant parts which are colorless with the upper polar out. They are mostly isotropic, but have varying amounts of material that is birefringent. Apparently, this indicates partial replacement of the organic matter by silica.

More precise x-ray determinations were performed on sludge using a powder camera. There are as few as five diffraction lines for low density, high organic fractions, and the number of lines increases in a regular pattern to 112 for the highest density low organic fraction. Table 5 shows the minerals that were found in the two sludges by x-ray and optical methods.

Willems et al. (1976) found a number of these same minerals in Copenhagen, Denmark, sludge and from chemical and x-ray analyses estimated the weight percentages for the various organic and inorganic components (Table 6).

Table 6. Estimated weight percentages of the organic and inorganic components of Copenhagen, Denmark, sewage sludge.

Component	Wt. % (dry)
Calcite	11
Gypsum	4
Non-crystalline calcium phosphates	8
Non-crystalline magnesium and zinc phosphates	3
Non-crystalline, hydrous aluminum and iron oxides	4
Quartz, feldspars, and clay	18
Organic matter and water (lost on heating to 450°C)	53

In the present study, a comparable amount of volatile material (53 wt. % - see Table 1) was found in the two sludges. We did not determine weight percentages

Table 5. Minerals in Dayton and Englewood sludges identified by x-ray and microscopic techniques.

Dayton	Englewood
Amesite - $2(Mg, Fe)O \cdot Al_2O_3 \cdot SiO_2 \cdot 2H_2O$	Calcite
Boracite (low-beta)- $Mg_3B_7O_{13}Cl$	Borickite
Borickite - $3CaO \cdot 7Fe_2O_3 \cdot 2P_2O_5 \cdot 24 \pm H_2O$	Garnet
Calcite - $CaCO_3$	Gypsum
Chalcopyrite - $CuFeS_2$	Oligoclase
Corrundum - Al_2O_3	Quartz
Diaspore - $AlO(OH)$	
Dolomite - $CaMg(CO_3)_2$	
Garnet - $(Mg, Fe, Ca)_3 (Al, Fe, Cr)_2 (SiO_4)_3$	
Goethite - $FeO(OH)$	
* Gypsum - $CaSO_4 \cdot 2H_2O$	
Hematite - Fe_2O_3	
Illite - $KAl_2(OH)_2[AlSi_3(O,OH)_{10}]$	
Ilmenite - $FeTiO_3$	
Magnetite - Fe_3O_4	
Oligoclase - $NaCaAlSi_3O_8$	
Pyrite - FeS_2	
Quartz - SiO_2	
Rutile - TiO_2	
Sphalerite - $Zn(Fe)S$	

* Actually, ahydrite (anhydrous $CaSO_4$) was identified in the sludge. However, the sludges were dried (100+°C) before weighing and centrifugation, and the water of the original gypsum was driven off. The diffraction pattern for gypsum was found in sludge that was not oven dried.

of the various inorganic components. However, in general, the Copenhagen sludge is similar to the Dayton sludge.

The mineralogy of the Englewood sludge is quite simple compared to that from Dayton. Dayton has a number of foundries which probably use sand, containing various heavy minerals, for forms. It appears that many, perhaps most, of the minerals have industrial sources.

Under the microscope, quartz, carbonate (calcite and dolomite), plagioclase feldspar (oligoclase), borickite, and garnet can be seen. The other minerals were identified from x-ray patterns. Borickite, amorphous, hydrated calcium oxide-iron oxide-phosphate was identified with the microscope. It is isotropic, with an index of refraction of about 1.58; is distinctly light tan with the upper polar out; and has conchoidal fracture (see Larsen and Berman, 1934). Willems et al. (1976 and Table 6) found two amorphous phosphates in Copenhagen sludge. They could be borickite or several other amorphous phosphates. Borickite makes up about 10 volume percent of whole Dayton sludge. It is noted that the Dayton and Englewood sludges contain 1.55 wt. % and 1.75 wt. % of phosphorous respectively (see Table 1). Willems, et al., report 2.58 wt. % phosphorous in the Copenhagen sludge. Clay (illite) was identified in several of the fractions from x-ray data. Optically, it is very difficult to estimate how much clay is present, because it is very intimately mixed with organic matter and possibly goethite in places.

Anhedral grains of quartz and carbonate are the most abundant crystalline materials in sludge. It appears that these minerals were formed prior to digestion. If they had been formed during the process, crystal faces would be present. In addition, amesite, corrundum, garnet, hematite, illite, ilmenite, magnetite, oligoclase, and rutile were probably formed before they entered the digester. However, the rest of the minerals may have formed during digestion.

Quartz and carbonate appear throughout the various density fractions, except for the very lightest (see Table 4). Grains of quartz (density - 2.65 gm/cm^3) and carbonate (calcite, density - 2.71 gm/cm^3 and dolomite,

density - 2.82 gm/cm^3) have organic matter and/or clay adhering to them. Apparently, this creates a buoyant effect which allows the minerals to be situated in centrifuge fractions having densities less than those for the pure minerals. A method will have to be found to completely remove clay and organic matter from mineral grains, before centrifugation. This would allow mineral grains to be trapped within their correct density liquid.

The proportion of carbonate becomes increasingly greater in the heavier fractions. For instance, in the 2.61 gm/cm^3 fraction, there is about 65 vol. % quartz and feldspar (density, 2.65 gm/cm^3 for both) and 20 vol. % carbonate. However, in the heaviest density fraction (2.89 gm/cm^3), carbonate makes up 65 vol. % of the sample and quartz and feldspar only 20 vol. %.

The most important question is: What components in the sludge contain the heavy metals? Tan et al. (1971) and others state that the heavy metals in sludge are complexed with organic material. However, Jenkins and Cooper (1964) suggested that the heavy metals in sewage sludge are present as insoluble sulfides or hydroxides. Dr. L. E. Sommers, Purdue University Agricultural Experiment Station, believes that some of the trace metals in sludge are in carbonate and sulfide compounds (personal communication, August, 1975). Page (1974) speaks of the inorganic forms of trace elements in sludge. However, he also states that, "presently, our knowledge of chemical forms of trace elements in sewage sludge is nil."

As was mentioned before, the greatest concentrations of heavy metals in the Dayton sludge are located in density ranges centered at 2.03 and 2.85 gm/cm^3 (see Figs. 5-6). Microscopic examination of a light density fraction (2.01 gm/cm^3) shows that 80-90 vol. % of the sample is organic matter (see Table 4). Ashing of a portion of the fraction gives a 57% loss of weight, which is believed to be due mainly to the burning of the organic matter. Under the microscope, the crystalline material is very fine-grained and difficult to identify. X-ray

analysis shows that quartz, calcite, amesite and garnet are present. It is noted that no clay, which could contain heavy metals, was found. Calcite is the only one of these minerals that would be dissolved in preparing the centrifuge fractions for chemical analysis. Therefore, it is the only crystalline material that could contribute trace elements. Calcite is much more abundant in the 2.61 gm/cm^3 (15-20 vol. %) fraction than in the 2.01 gm/cm^3 ($\lesssim$1.0 vol. %) fraction, but the 2.61 gm/cm^3 fraction has minimum trace element contents (see Table 4 and Figs. 5-6). Thus, it appears that calcite does not contribute substantial amounts of trace elements to the 2.01 gm/cm^3 fraction, and it is concluded that the trace elements in this fraction are contained primarily in organic matter.

In a high density fraction (2.89 gm/cm^3) which is mostly crystalline (5.2 wt. % volatiles, see Table 4), the heavy metals are probably contained in one or more of the minerals. In addition to quartz, calcite, amesite, and garnet, there is dolomite, hematite, goethite, magnetite, corrundum, gypsum (anhydrite, see Table 5), boracite, ilmenite, rutile, pyrite, chalcopyrite and sphalerite. As with the lighter density fraction, no clay was identified. Again, considering the sample preparation process, only boracite, the carbonates, the sulfides and gypsum-anhydrite could supply the trace elements. The low trace element fraction (2.61 gm/cm^3, see Figs. 5-6 and Table 4) contains clay and the minerals that are in the 2.89 gm/cm^3 fraction except gypsum-anhydrite, goethite, and sphalerite. Since goethite is probably not dissolved, the sulfate and sulfide minerals are the most likely candidates as hosts for the trace heavy metals.

As mentioned before, 75% of all the trace elements in the two sludges are in the low density, high organic content fractions, and are most probably restricted to the organic material itself. Some of the trace elements appear to be in inorganic compounds (minerals). In the Dayton sludge, only about 10 wt. %

of the total sample occurs in the high density range centered at 2.85 gm/cm^3 (see Fig. 3). The trace elements are located in only a few minerals which probably make up less than 10 wt. % of the high density material. Therefore, the total amount of trace elements from inorganic sources is very small, and it is concluded that most of the trace heavy metals in the sludges analyzed in this study are in organic compounds.

X-ray and microscopic investigation of Miami silt loam soil

Since many of the density fractions for Miami silt loam soil were so small, fractions were grouped in order to have enough material for accurate chemical analysis. Table 7 gives the density ranges, weight percent volatiles, and minerals present. X-ray powder films show 42 diffraction lines for the low density fractions (1.60-1.99 gm/cm^3). The number of lines increases to 105 for the high density fractions (2.60-2.87 gm/cm^3). There is an orderly decrease in weight percent volatiles from 45 wt. % in the low density material to 2.0 wt. % in the high density material.

Unfortunately, the organic and inorganic material are so intimately mixed that in some of the fractions, it is very difficult to distinguish them optically. As with sludge, many of the mineral grains are coated with a mixture of clay and organic matter. This causes a buoyant effect, and many of the mineral grains are in fractions having densities lower than those of the pure minerals.

Organic matter appears in the same three forms found in sludge. Partly silicified plant parts occur in all but the heaviest fractions. They make up about 15 vol. % of the lighter fractions and decrease in amount in the heavier fractions. Quartz, feldspar and carbonate are easily identified in the heaviest fraction (2.87 gm/cm^3). Quartz and feldspar make up about 90 vol. %, and carbonate one to two volume percent.

TABLE 7. DENSITY - COMPOSITION OF SELECTED MIAMI SILT LOAM DENSITY FRACTIONS

Fraction Density Range (gm/cm^3)	Weight % Volatiles	Minerals Present									
		quartz	montmorillonite	oligoclase	calcite	dolomite	borickite	boracite	amesite	pyrite	garnet
1.60-1.99	45	X	X								
2.00-2.19	28	X	X	X							
2.20-2.39	18	X	X	X	X						
2.40-2.49	11	X	X	X	X	X	X				
2.50-2.59	6	X	X	X	X	X	?		?		
2.60-2.87	2	X	X	X	X	X	X	X	?	X	X

SUMMARY AND CONCLUSIONS

Sewage sludge and soil can be separated into component density fractions with zonal centrifugation, using tetrabromoethane-ethanol as gradient material. Up to 75 weight percent of total sludge samples has relatively low density, from 1.60 to 2.10 gm/cm^3. Eighty weight percent of the total soil sample falls within 2.50 and 2.90 gm/cm^3. Sludge contains from 95 weight percent volatiles in low density fractions to less than 10 weight percent in high density fractions. Soil contains from 45 weight percent volatiles in low density material to 2.9 weight percent volatiles in high density material. It is believed that percent volatiles is a measure of the amount of organic material.

Distribution of trace amounts of cadmium, copper, lead, nickel, and zinc with respect to density in sludge and soil shows curve-like, continuous trends. This implies that more than random scattering accounts for the distribution. Two different narrow density ranges in Dayton sludge contain the greatest concentrations of trace elements. One is centered at 2.05 gm/cm^3 (high organic matter) and the other is centered at 2.85 gm/cm^3 (low organic matter). Seventy-five weight percent of the total trace elements in sludge is contained in organic-rich fractions. A strong, positive correlation exists between trace element concentration and percent volatile material in soil. A relatively small amount of the trace elements in sludge appears to be associated with minerals, particularly sulfides and sulfates, in high density fractions. Twenty different minerals were found in sludge and soil, by x-ray diffraction analysis and microscopic investigation.

In view of the fact that a high weight percentage of the trace elements in sludge and soil are associated with organic matter, it is concluded that most of the trace heavy metals in sludge and soil analyzed in this study are in organic compounds. The trace element distributions suggest that particular organic

compounds, within a limited density range, contain the greatest concentrations of trace elements. Infrared spectrographic analysis suggests the presence of two or more carboxylate anions in sludge. It is believed that they are the most likely hosts for the trace heavy metals.

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MOVEMENT OF TRACE ELEMENTS IN SEWAGE SLUDGE-AMENDED SOIL COLUMNS

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ABSTRACT

Maximum and twice maximum allowable amounts of Dayton, Ohio, municipal sewage sludge were applied to columns of Miami, a clay-rich soil and Fox, a sand-rich soil, in the laboratory. Deionized water was added to the tops of the columns over a ten-month period to simulate an approximately two-year rainfall. Layers of the soil were analyzed for trace amounts of cadmium, copper, lead, nickel and zinc. It appears that ions of the metals percolate to the same depth with both dosages. Ions in Miami soil do not percolate downward more than 15 cm, and ions in Fox soil do not percolate more than 30 cm. Assuming that pH and other parameters of the soils remain constant, it does not appear that applying Dayton sludge to local soils poses a serious threat to ground water purity.

INTRODUCTION

In recent years, municipalities have been plagued with the problem of disposal of sewage sludge. Rising land costs make lagooning less practical. Energy costs and pollution guidelines discourage burning. Application of sludge as a soil conditioner and low grade fertilizer on farm land is an attractive alternative. Although sludge contains nutrients such as nitrogen, potassium and phosphate, it also contains toxic heavy metals. When applied to soil, sludge breaks down and releases many of these trace elements into the soil system. Once released, these elements could be taken up by plants, thus entering the food chain, or they might percolate downward into the ground water zone. In areas, such as in and around Dayton, Ohio, all water is drawn from underground sources. Therefore, it is of great importance to determine how far down heavy metals, leached from sewage sludge, move and whether ground water is contaminated. This study addresses itself to the questions: (1) how far down do ions of cadmium, copper, lead, nickel and zinc leached from sewage sludge move in a soil column? and (2) can groundwater be polluted?

MATERIALS AND METHODS

Two very different major soils of the Dayton, Ohio, area were used: (1) the Miami, a silty, clay loam formed on glacial till, the most abundant agricultural soil in the area; and (2) the Fox, a sandy clay loam formed on glacial outwash. Table 1 gives some of the physical and chemical characteristics of these soils and Dayton municipal sewage sludge used in this study. It is interesting to note that Lund et al. (1976) found comparable background amounts of cadmium, nickel, copper and zinc in a Delhi series loamy sand soil from California (see Table 1).

TABLE 1. SOME OF THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF MIAMI AND FOX SOILS AND DAYTON SEWAGE SLUDGE

Characteristics	Dayton sewage sludge	Miami soil	Fox soil	Delhi series loamy sand soil from California*
Sand content		11%	64%	
Silt content		48%	14%	
Clay content		41%	22%	
pH	6.34	5.51	6.32	
Cation exchange capacity (C.E.C.)	20.8 meq/100g	22.5 meq/100g	15.8 meq/100g	
	Trace heavy metal content (ppm)			
Cd	1413	0.7	.14	.4 - .5
Pb	3230	21.1	10	- -
Ni	654	18.0	10	10 - 12
Cu	2150	10.2	9.4	10 - 13
Zn	11100	48	39	35 - 40

* Lund et al. (1976)

Intact cores of the two soils were obtained by driving two feet sections of four inch diameter PVC-DWV pipe into the ground. Care was taken to insure that the soil was disturbed as little as possible, in order to retain field conditions in the laboratory. The soil was kept in the plastic pipes. These assemblages served as soil columns for the percolation studies.

Undigested (primary) liquid sewage sludge was anaerobically digested in the laboratory. During the process, $CdNO_3$ was added to the sludge to give it a total Cd concentration of 1413 ppm. This digestion process is described on page 31 of this report. The liquid sludge was applied to the tops of columns in treatments equal to the maximum (1X - treatment) and twice maximum (2X - treatment) amounts proposed by the Ohio Agricultural Research and Development Center (Grooms, 1975). The sludge dosages are determined by taking into consideration the C.E.C. of the soil and the heavy metal concentrations of the sludge. The recommended applications hold for a soil with pH less than 6.5. The "maximum amount" refers to the total sludge that can ever be applied to the soil. The calculations used to determine application amounts in this study are similar to those shown on page 81 of this report. Table 2 shows the amounts of sludge that can be applied in the field and the scaled down amounts applied to the tops of soil columns. The lab digested sludge contains 3.1% solids. Table 2 gives the total amounts of liquid sludge applied to the Miami and Fox soil columns. The sludge was applied gradually in approximately 200 ml increments.

TABLE 2. AMOUNTS OF DAYTON SEWAGE SLUDGE APPLIED TO MIAMI AND FOX SOILS

	Maximum amount of sludge allowed			Total amount of liquid sludge applied (3.1% solids)	
soil	Short tons (dry) / acre	Metric tons (dry) / hectare	Gms. (dry) / cm^2	Maximum treatment 1X (ml)	Twice maximum treatment - 2X (ml)
Miami	21.1	47.3	0.47	1240	2480
Fox	14.9	33.4	0.33	871	1742

The Dayton area receives an average of 36 inches of rain per year. The rainwater has an average pH of 4.8. To reproduce twice the field rainfall on the columns, 250 ml increments of deionized water (pH=4.8) were added each week for 10 months, giving 20 months simulated time.

After drying, the columns were dismantled. Layers of soils 2.5 cm thick were scraped from the columns. Each layer was ground, mixed very thoroughly and sieved to -20 mesh. A sample for trace element analysis by atomic absorption spectroscopy was selected from each layer. The samples were treated and analyzed following the procedures described on pages 95-102 of this report.

RESULTS AND DISCUSSION

Tables 3 and 4 give the trace element values for the different layers of the two soils. Figure 1 (a-e) shows plots of the concentrations of the various trace elements (log scale) versus depth of downward percolation. Background values which are quite uniform with depth, except for lead (Fig. 1d.) are indicated. The variation of lead background probably reflects the absorption by the soil of lead from gasoline engine exhaust gases. It is interesting to note that, although the lead background values for Fox soil (14.1- 9.6 ppm) are less than those for Miami soil (25.3 - 14.8 ppm), the pattern of decrease in concentration with depth is similar for both soils. The two sample sites are fourteen miles apart.

With both 1X and 2X treatments on Miami soil, there does not seem to be much downward movement of heavy metals below 15 cm. For some reason, with the possible exception of Cu, the metal values resulting from 2X treatments decrease more rapidly with depth than do those from 1X treatment. However, with both treatments, metal values are very similar when they reach or closely approach background levels, at 15 cm.

With both 1X and 2X treatments on Fox soil, there does not seem to be much downward movement of heavy metals below 35-40 cm. Trace element movement patterns

TABLE 3. TRACE ELEMENT VALUES IN LAYERS FROM MIAMI SOIL COLUMN (ppm)

Soil depth (cm)	Zn	Cu	Ni	Cd	Pb
			Miami 1X treatment		
2.5	282	39.4	41.1	27.4	87.8
5.0	163	26.9	26.5	15.3	51.4
7.5	124	19.3	21.7	9.2	36.5
10.0	71	10.8	17.9	2.4	19.8
12.5	58	12.2	19.0	1.5	18.9
15.0	55	9.6	17.7	1.9	17.9
Control (aver. for all depths)	48	10	18	0.7	21
			Miami 2X treatment		
2.5	393	67	59	41	94
5.0	110	21	23	6.6	23
7.5	72	19	18	2.3	15
10.0	61	14	17.6	1.7	16
12.5	57	13.5	17.5	1.1	17
15.0	56	8.6	17.1	1.1	15
Control (aver. for all depths)	48	10	18.0	0.7	21

TABLE 4. TRACE ELEMENT VALUES IN LAYERS FROM FOX SOIL COLUMN (ppm)

Soil depth	Zn	Cu	Ni	Cd	Pb
			Fox 1X treatment		
2.5	145	21	19	16	51
5.0	73	12	13	4.7	25
7.5	66	9.8	11	2.3	17
10.0	55	9.2	13	3.4	20
12.5	50	8.7	12	2.1	19
15.0	47	9.2	11	.9	13
15.0 - 20.0	45	-	10	1.7	15
20.0 - 25.0	50	-	10	1.1	15
25.0 - 30.0	41	-	10	.5	13
30.0 - 35.0	40	-	10	.1	11
35.0 - 40.0	-	-	-	0.0	9.8
Control (aver. for all depths)	39	9.4	10	.14	10
			Fox 2X treatment		
2.5	136	26	20	13	41
5.0	79	14	16	5.8	31
7.5	70	11	15	5.1	27
10.0	63	8.7	15	3.3	23
12.5	61	9.6	14	3.8	19
15.0	61	9.0	13	3.0	19
15.0 - 20.0	59	-	13	3.7	21
20.0 - 25.0	48	-	13	1.3	17
25.0 - 30.0	45	-	11	.5	16
30.0 - 35.0	41	-	10	-	14
35.0 - 40.0	-	-	-	-	-
Control (aver. for all depths)	39	9.4	10	.14	10

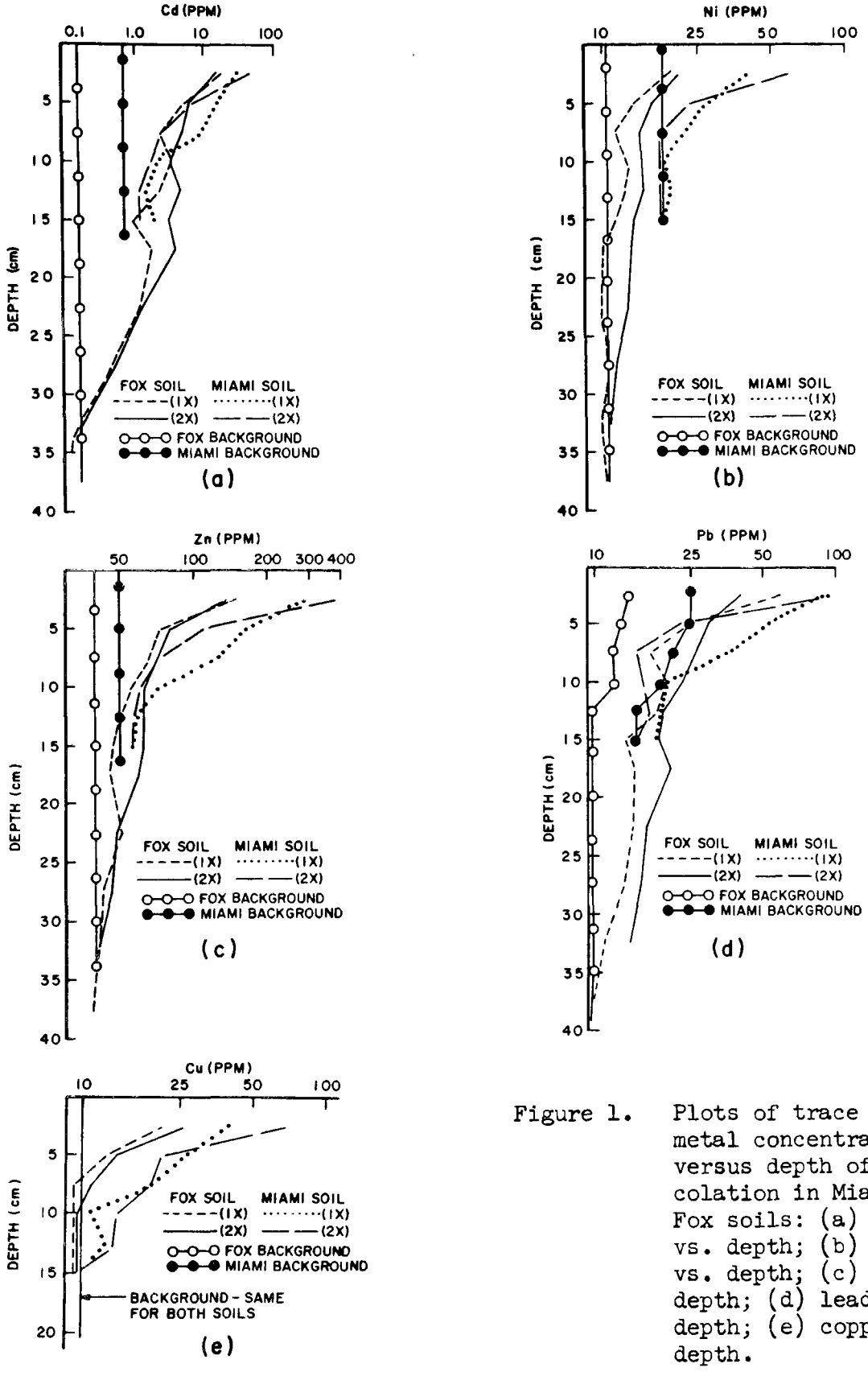

Figure 1. Plots of trace heavy metal concentrations versus depth of percolation in Miami and Fox soils: (a) cadmium vs. depth; (b) nickel vs. depth; (c) zinc vs. depth; (d) lead vs. depth; (e) copper vs. depth.

for both treatments are very similar except for copper (Fig. 1e) and nickel (Fig. 1b). With both treatments, copper values reach background by 10 cm, and with 1X treatment, nickel values reach background between 15-20 cm. Lead values (Fig. 1d) for 2X treatment did not reach background (10 ppm) at 30-35 cm, and lead ions may percolate below 40 cm. However, they probably would not move down much farther, since values at 30-35 cm are near background.

In comparison to the results presented here, Boswell (1975) found movement of zinc to 30 cm and lead, copper, cadmium and chromium to 15 cm in an acid soil which had been amended with 16.8 metric tons/ha of sewage sludge. Page (1974) analyzed data reported by Andersson and Nilsson (1972) and states that, "practically all of the Mn, Zn, Cu, Ni, Co, Cr, Pb, Cd, Mg, As, and Se remained in the surface 20 cm of soil following application of 84 metric tons/ha of sewage sludge over a 12-year period." Hinesly et al. (1972) suggest that zinc, lead, copper, cadmium, nickel and chromium percolate below 15 cm in soil amended with 44 to 166 metric tons/ha of sewage sludge over a period of three years.

It is expected that metals would percolate further down in Fox than in Miami soil. Fox soil has a lower C.E.C., because of a lower clay content and a much higher sand content making it more porous and permeable than Miami soil (see Table 1). This would also explain why the top sections of Fox soil contain lower amounts of trace elements than comparable sections in Miami soil. The same amounts of metals would flow through the two columns, but the ions would move downward faster and be spread out over a greater vertical distance in Fox soil.

It appears that the depth to which heavy metal ions percolate is more dependent upon the nature of the soil than on the amount of metals applied. In spite of the extremely high concentrations of Cd (1413 ppm), Cd ions did not percolate further than the others.

In addition to the above mentioned factors, organic matter (O.M.) which

chelates heavy metals, also affects their percolation and distribution. Percent volatiles which approximates O.M. content was determined for the layers in the Miami soil column. Values decrease in a linear fashion from 5.2% in the 2.5 cm layer to 3.6% in the 15 cm layer. Soil pH is another factor which controls metal ion movement. Solubility of metal ions in the soil system is directly dependent upon pH. The two soils used in the present study are weakly to moderately acid (see Table 1). Perhaps, if the pH's had been closer to neutrality or even basic, heavy metal percolation may have been less.

CONCLUSIONS

In Miami, a clay-rich soil with a pH of 5.51, amended with allowable amounts of Dayton municipal sewage sludge, ions of cadmium, copper, lead, nickel and zinc do not percolate much below 15 cm in about a two-year period. In Fox, a sand-rich soil, with a pH of 6.32, amended with allowable amounts of Dayton municipal sewage sludge, these same ions do not percolate much below 40 cm in about a two-year period. Considering the results of other workers, longer periods of application may not significantly alter the situation.

Except for floodplain areas close to streams, the ground water table in the Dayton area is considerably lower than 30 cm. The minimum depth in most of the area would probably not be less than one meter. However, under extreme conditions, such as extraordinary amounts of rain, it could rise higher. Additional studies should be made in the Dayton area, applying sludge over longer periods of time, in the field. However, based on the datapresented here, it does not appear that application of Dayton sludge in recommended amounts to local soils poses a serious threat to groundwater purity.

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